No. 23-2227

United States Court of Appeals for the Federal Circuit

BRUKER CELLULAR ANALYSIS, INC.,

Appellant,

v.

UNIVERSITY OF BRITISH COLUMBIA,

Appellee.

Appeal from the United States Patent and Trademark Office,
Patent Trial and Appeal Board in No. IPR2021-01249
Administrative Patent Judges Kalan, Kaiser, and Ogden

OPENING BRIEF OF APPELLANT BRUKER CELLULAR ANALYSIS, INC.

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PATENT CLAIMS AT ISSUE

U.S. Pat. No. 10,087,408 B2, Claim 1 (Appx128)[1]

1. A method of culturing a cell, the method comprising:

retaining the cell at a retaining position within an individual chamber of a microfabricated device;

perfusing the cell with a perfusion fluid by flowing the perfusion fluid into the individual chamber through an inlet and out of the chamber through an outlet,

wherein the outlet is positioned such that gravitational forces acting on the cell to keep it at or near the retaining position exceed hydrodynamic forces acting on the cell to move it toward the outlet;

culturing the cell within the chamber and monitoring a response in the chamber; and

selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step.

[1] In reaching its Final Written Decision, the Board did not address, nor did UBC dispute, Bruker Cellular's demonstration that the additional limitations of challenged dependent claims 6, 11, 16, 19, 24, 26, 27, and 30 were disclosed by one or both of the primary references Dimov and Park. The dependent claims are not included here for that reason.

CERTIFICATE OF INTEREST

Counsel for Appellant Bruker Cellular Analysis, Inc. (formerly known as PhenomeX Inc. and before that Berkely Lights, Inc.) certify the following:

1. The full name of every entity represented by undersigned counsel in this case is:

   Bruker Cellular Analysis, Inc.

2. The name of the real party in interest for every entity represented by undersigned counsel in this case is:

   None/Not Applicable

3. The full names of all parent corporations and publicly held companies that own 10 percent or more of the stock of the entities represented by undersigned counsel in this case are:

   Bruker Corporation is the publicly held parent corporation of Appellant Bruker Cellular Analysis, Inc.

4. The names of all law firms, partners, and associates that (a) appeared for the entities in the originating court or agency or (b) are expected to appear in this court for the entities are:

Irell & Manella LLP: Michael R. Fleming, Andrew Krause

5. The title and number of any case known to be pending in this or any other court or agency that will directly affect or be directly affected by this court's decision in the pending appeal are:

AbCellera Biologics Inc. et al. v. Bruker Cellular Analysis, Inc. f/k/a Berkeley Lights, Inc., Case No. 3:20-cv-08624-JST (VKD) (N.D. Cal.) (transferred from C.A. No. 20-cv-1230-RGA (D. Del.))

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STATEMENT OF RELATED CASES

No appeal from the *inter partes* review proceedings below was previously before this or any other appellate court.

The outcome of this appeal may impact a co-pending district court case in which the University of British Columbia along with AbCellera Biologics, Inc. have accused Bruker Cellular of infringing the patent at issue in this appeal: *AbCellera Biologics Inc. et al. v. Bruker Cellular Analysis, Inc. f/k/a Berkeley Lights, Inc.*, Case No. 3:20-cv-08624-JST (VKD) (N.D. Cal.) (transferred from C.A. No. 20-cv-1230-RGA (D. Del.)).

I. INTRODUCTION

None of the limitations of claim 1 of U.S. Pat. No. 10,087,408 were new. The patent relates to culturing cells in a microfluidic device. Each of the limitations of claim 1 was known to a person having ordinary skill in the art and found within the prior art submitted in the proceeding below. Using a microfluidic device to isolate, culture, and monitor cells was not new as of the priority date of the '408 patent. This idea is reflected in the first four limitations of claim 1, and patentee UBC does not contest that prior art references Dimov and Park each disclose all four.

Removing a particular cell of interest from a microfluidic device (as reflected in the last limitation of claim 1), which was the focus of the Board's decision below, was also not new as of the priority date of the '408 patent. In fact, it was a known need in the art—one described in the Kovac reference as "ubiquitous." Selective cell removal was necessary to isolate particularly interesting cells for further investigation. With this motivation, a person having ordinary skill in the art ("POSA") would have applied known techniques for selectively recovering cells from the microfluidic devices described in Dimov and Park, with a reasonable expectation of success. The challenged claims were therefore obvious in view of the prior art.

The Board erred in finding the challenged claims were not shown to be unpatentable because it conflated "motivation to combine" and "reasonable likelihood of success" in its analysis. The Board weighed "whether there was a sufficient motivation and sufficient background knowledge in the art, at the time of the claimed invention, that a person of ordinary skill in the art would have modified [the prior art devices] to introduce selective recovery" as recited in the final limitation of claim 1. Appx25; *see also* Appx48; Appx58-59; Appx70. The Board stated that it did ***not*** rely on whether Bruker Cellular "had shown a reasonable expectation of success." Appx80. Consequently, the Board's decision was based entirely on whether there was a motivation to modify Dimov and Park to selectively recover a cell from their devices. That is where the Board erred.

First, the Board legally erred when it relied only on supposed difficulties with applying particular cell removal and recovery techniques to Dimov's trenches and Park's wells when assessing whether a POSA would have been motivated to modify their devices in the first instance. "Motivation to combine" and "reasonable expectation of success" are separate and distinct requirements of obviousness, and evidence showing motivation to combine is broader than evidence that goes to the reasonable expectation of success.

Second, the Board legally erred when it required Dimov's and Park's teachings themselves to provide the motivation to selectively recover cells. The

motivation to modify or combine a reference need not come from the primary reference itself.

Third, the Board's finding that there was no motivation to modify Dimov and Park to include selective recovery was not supported by substantial evidence. The prior art is replete with express motivations to recover a cell or cells of interest from a microfluidic device in which experiments identify cells of interest. For example, Kovac stated that the "need to isolate small numbers of specific cells from background populations is ubiquitous" and, in cell biology experiments, cell isolation "can be a tool to analyze the results of an experiment and isolate particularly interesting cells for further investigation." Appx899 (cited in Appx706). Even the '408 applicants admitted that cell recovery was "often" required "to select cells of interest for larger scale culture." Appx125(26:1-6); Appx1350(40:5-6). It is only common sense that a POSA conducting experiments on cells would be motivated to recover a particular cell having interesting properties or behaviors. Thus, a POSA would have been motivated to improve Dimov and Park with known selective cell recovery techniques. Such techniques included the standard lab practice of pipetting (as known in the art and taught by Kovac and Han); laser levitation (as taught by Kovac), and other cell-manipulation techniques (as taught by Dimov).

In considering whether a POSA would have been motivated to make those improvements, the Board consistently relied on evidence and argument directed to reasonable expectation of success, which is legally erroneous. Additionally, the Board committed legal error when it found Han (which disclosed a selective recovery technique) did not reflect the background knowledge of a POSA.

II. JURISDICTIONAL STATEMENT

The Board had jurisdiction under 35 U.S.C. §§ 6(b)(4) and 311, and entered its final written decision on January 19, 2023. Appx1-72. Bruker Cellular timely requested rehearing on February 21, 2023 (Appx635-654), and the Board denied rehearing on July 20, 2023. Appx73-88. Bruker Cellular timely appealed on July 26, 2023. Appx655-658. This Court has jurisdiction under 28 U.S.C. § 1295(a)(4)(A) and 35 U.S.C. §§ 141(c), 144.

III. STATEMENT OF THE ISSUES

1. Whether the Board committed legal error when it found there would have been no motivation to modify Dimov or Park because of purported difficulties in making specific modifications to the references, which speaks to whether there would have been a reasonable expectation of success, and not to motivation to combine.

2. Whether the Board committed legal error when it required the motivation to modify the Dimov and Park references to be found in the references themselves.

3. Whether the Board's finding there was no motivation to modify Dimov and Park to include selective recovery was unsupported by substantial evidence, where the need to recover a cell or cells of interest was "ubiquitous" in the art and a POSA would have been motivated to use any a variety of selective cell recovery techniques to meet that need, and because the Board committed legal error when it found Han did not reflect the background knowledge of a POSA.

IV. STATEMENT OF THE CASE

The '408 patent is in the crowded field of cell culturing in microfluidic devices. For decades, scientists have worked on ways to manipulate and study separate cells for purposes ranging from in vitro fertilization to antibody discovery to stem cells to disease modeling. Against that background, the '408 patent claims a four-step process of (1) retaining a cell in a chamber of microfluidic device, (2) perfusing the cell (with a suitably located outlet for the perfusion fluid so the cell is kept in place by gravity), (3) culturing and monitoring the cell, and (4) selectively recovering the cell based on the monitoring. Patentee UBC concedes all of the steps may be found in the prior art and all but the last step are found in the asserted primary references. This appeal is only about whether a scientist working on microfluidic cell culturing would have been motivated to take a cell out of their microfluidic device for further study.

A. The Asserted References

Four references are relevant on appeal: Dimov (Appx762-793),[2] Park (Appx893-898), Kovac (Appx899-908), and Han (Appx909-915).

1. Dimov

Dimov is U.S. Pat. No. 8,906,669 B2, issued from application PCT/EP2009/063229 filed in 2009, and is titled "Microfluidic Multiplexed Cellular and Molecular Analysis Device and Method." Appx762-793. The application was published internationally as WO 2010/040851 A2 in 2010 (Appx794-858) and published in the United States as US 2011/0262906 in 2011. Appx859-892.

Dimov describes a microfluidic multiplexed cellular and molecular analysis system and various methods of using it. Appx141. The Dimov system comprises one or more microfabricated capture chambers, each exemplified by the schematic in Figure 4B below. Appx766(Fig. 4B). Figure 4B shows a fluid path leading to a capture chamber, here a trench, to capture "particles" such as cells, which fall down from the flowing fluid to the bottom of the trench under the influence of gravity when the fluid slows down as it enters the trench:

[2] For convenience, citations to "Dimov" herein are made to U.S. Pat. No. 8,906,669 B2 (Appx762-793), but the cited portions can also be found in WO 2010/040851 A2 and US 2011/0262906.

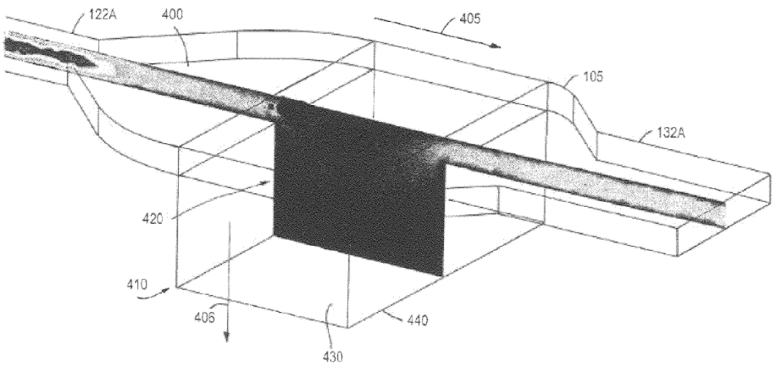


FIG. 4B

Later, Hansen et al. filed the application that would ultimately issue as the '408 patent. Appx89. Like Dimov, the '408 patent describes a microfluidic system for cell culture and includes a figure that is strikingly similar to the schematic of the Dimov device that came before it. Appx95(Fig. 4). Below are the two figures side-by-side (Appx142):

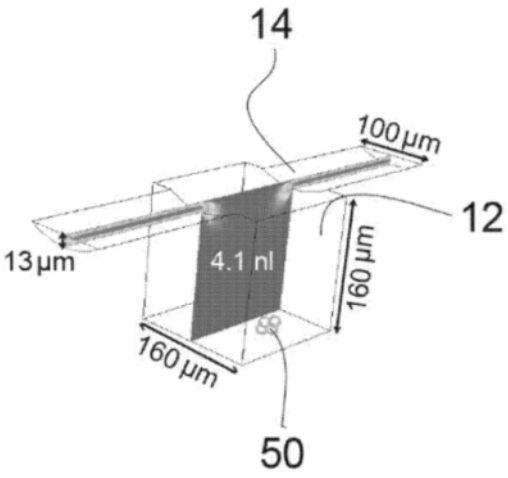


FIGURE 4

Hansen et al.

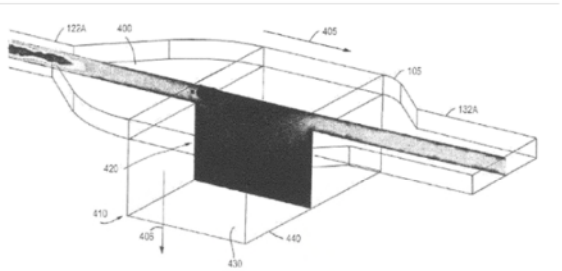


FIG. 4B

Dimov et al.

Dimov explains that once captured, the cells are retained in the trench, by dimensioning the trench relative to the flow rate of the fluid. Appx786(6:44-47);

Appx787(7:9-12). "By retaining the cell within a capture chamber or trench and then simply flowing different fluids past that captured cell, it is possible to achieve capture, labeling and analysis within a single structure." Appx788(10:55-58). The device shown in Figure 4B may be configured with other devices in array structures, all formed on a single monolithic device, as illustrated in Figure 1:

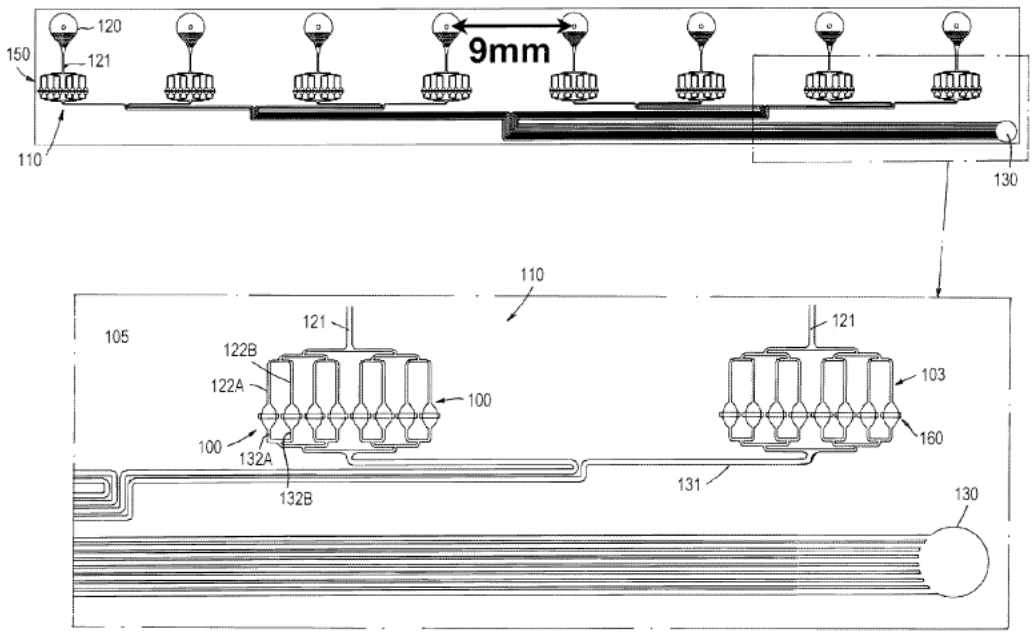


FIG. 1

Appx763(Fig. 1). Figure 1 shows collection of arrays, where

> Each array 110 in this configuration comprises eight identical devices 100, sharing a common input 120 and a common output 130. The common input branches into 8 feed lines 122*a*, 122*b*, 122*c* etc., provided upstream of capture chambers for each device respectively. Each device has a dedicated waste line 132*a*, 132*b*, 132*c* etc., provided downstream of the capture chamber and configured to distribute fluid out of the devices into the common output 130.

Appx785(4:1-8).

Dimov further explains that the "capture region provides an effective experimental region wherein a capture cell can be stimulated or modified by suitable experimental techniques. By changing the fluid that is introduced in the device, captured cells can be exposed to different environments and their responses can be tested . . . ." Appx787(7:34-39). One example of monitoring cellular responses in the capture chamber disclosed in Dimov is "real-time protein analysis whereby it is possible to monitor live cell interactions with stimulation agents and/or other cells and in real time detect with high specificity the expression of surface proteins." Appx790(13:60-63). Figure 23 is an example in which

> J774 macrophages 2300 were activated with LPS (200 ng/ml) in the positive control case, while in the negative control no LPS was present in the culture medium. As the stimulated macrophages began to express the CD86 proteins on the cell surface, the fluorescent CD86 antibodies generated a fluorescent signal from the cell surface.

Appx790(14:38-43). A variety of experiments can be performed in Dimov's capture chambers. Appx790(13:16-22).

Dimov also teaches that it is possible "to provide for subsequent movement of the particles—either within the trench so as to provide for mixing or the like, or to effect removal of the particles out of the trench." Appx789(11:24-26). Dimov expressly teaches that these "particles" can be cells. Appx787(7:4-5, 7:8-9); *see also id.* at Appx784(1:57) (highlighting that "the particles are cells" in an embodiment). Dimov explains "[s]uch arrangements will typically require a

capacity to manipulate the particles and this can be conducted either before or subsequent to capture of the particles within the trench." Appx789(11:27-30). Dimov identifies a variety of techniques for removing a particle, including a cell, from a trench:

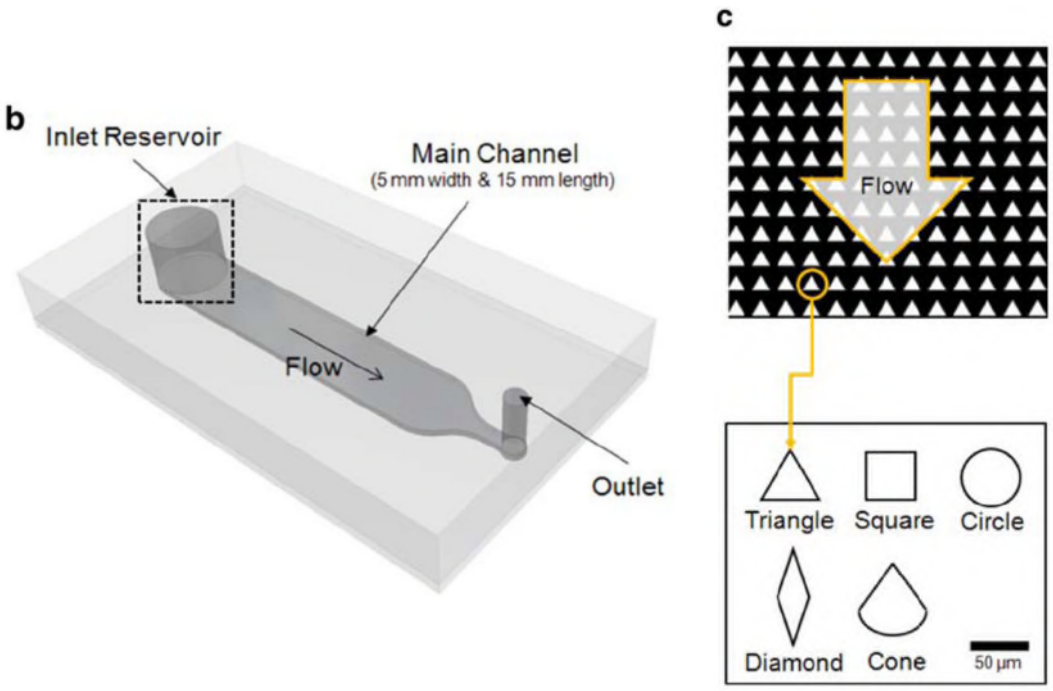
> Acoustic
> Magnetic
> Inertial
> Electric
> Dielectrophoretic
> Thermo-hydrodynamic
> Laser tweezers
> Hydrodynamically induced agitation
> Specific or unspecific attachment to surface.

Appx789(11:30-40).

2. Park

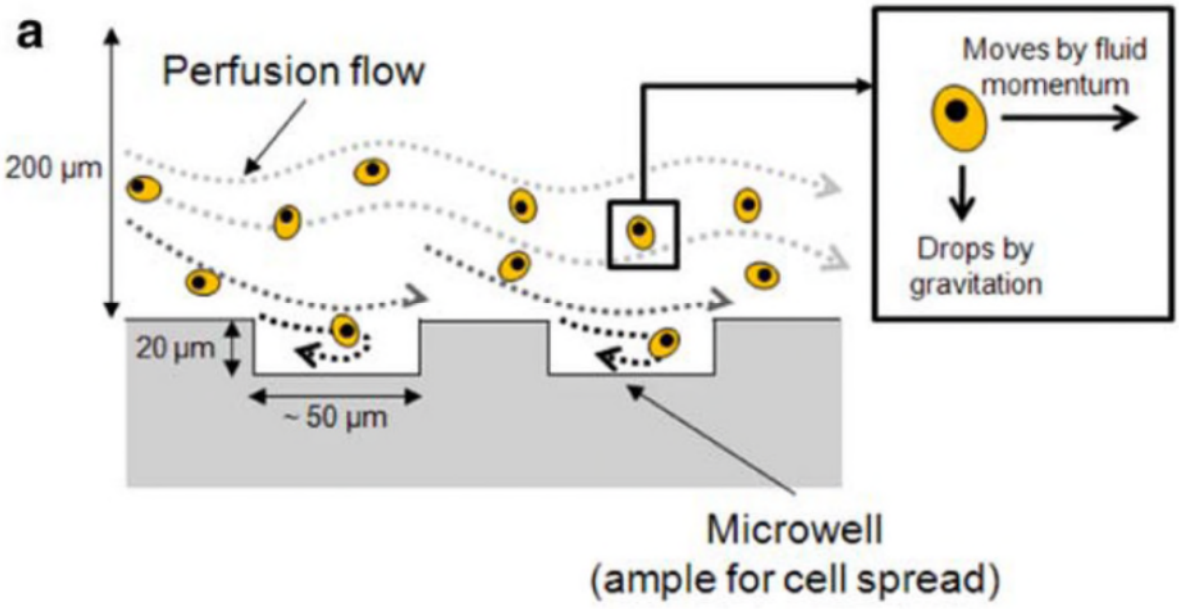
Park is a journal article entitled "Single Cell Trapping in Larger Microwells Capable of Supporting Cell Spreading and Proliferation," published online in 2009. Appx893-898.

Park discloses a device that "consists of a microwell array that covers the bottom of the chip, and a long narrow channel . . . that guides fluid flow over it." Appx895. The overall system geometry is illustrated in Fig. 1b, on the left, below:



Appx894. Park's system consists of four parts: "the inlet reservoir where cell suspension is introduced, [the] main channel, [the] microwells . . . patterned on the bottom surface of the main channel, and the outlet which is connected to a pulling syringe pump." Appx894. An array of triangular microwells is illustrated in Fig. 1c, on the right, above. The microarray, under the main channel, is a "50 x 200 array of triangular microwells (typical side length dimension of 50 µm with depth of 20 µm) [that] extends downward into the bottom layer giving a total of 10,000 microwells." Appx895.

During operation of Park's device, a cell suspension enters the microchip, flows through the channel, and cells settle into the microwells, where they "are caught," as shown in Fig. 1a below. Appx894-895.



In Park's system, "[a]s a typical cell flows through a microchannel, it will gradually settle toward the bottom surface due to gravity (Fig. 1a)." Appx895. Park explains that "[d]epending on the velocity of the cell and its position relative to the micro-wells, a cell may approach a threshold slow speed at which point gravity shifts the cell into a lower streamline that leads into a recirculation zone in the microwell." Appx895. Park further teaches that "[r]ecirculation is a unique flow phenomenon that effectively captures and traps particles in this zone." Appx895.

Park also teaches that this device is useful for both cell culturing and performing experiments: "[C]ell culture is possible due to the ample space of the microwells (Fig. 3c) allowing investigation of both long-term cell responses and instantaneous cell reactions." Appx898. For example, Park reports data showing

that "[a]fter 2 days of culture, single or multiple cells (DsRed-transfected PC3 cells) were observed in microwells . . . ." Appx897(Fig. 3c caption).

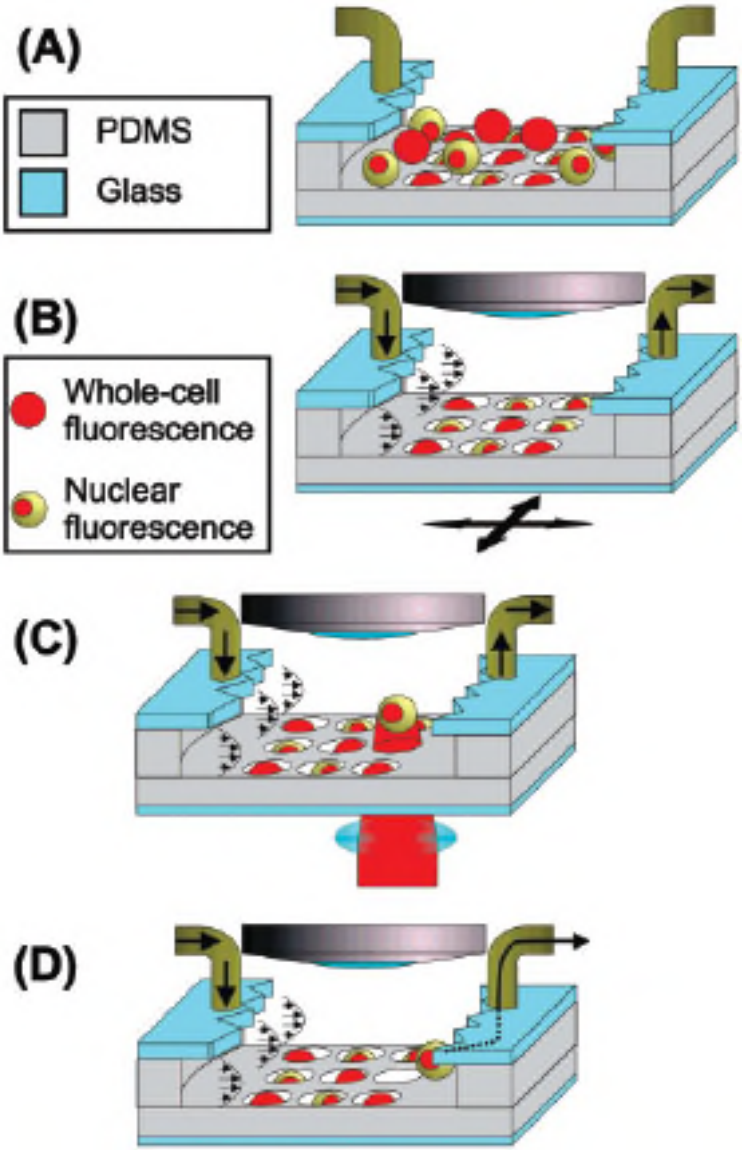
3. Kovac

Kovac is an article entitled "Intuitive, Image-Based Cell Sorting Using Optofluidic Cell Sorting" and was published in 2007. Appx899-908. Kovac states that the "need to isolate small numbers of specific cells from background populations is ubiquitous, with applications in pathology, clinical diagnosis, cloning, and cell biology research." Appx899. For cell biology experiments, Kovac identifies a need to "select a desired starting population of cells of known characteristics" or "isolate particularly interesting cells for further investigation." Appx899. Kovac's abstract neatly summarizes the disclosed structures and techniques developed to satisfy that need:

> We present a microfluidic cell-sorting device which augments microscopy with the capability to perform facile image-based cell sorting. This combination enables intuitive, complex phenotype sorting based on spatio-temporal fluorescence or cell morphology. The microfluidic device contains a microwell array that can be passively loaded with mammalian cells via sedimentation and can be subsequently inspected with microscopy. After inspection, we use the scattering force from a focused infrared laser to levitate cells of interest from their wells into a flow field for collection.

Appx899. Similar to the methods described in Dimov, Kovac teaches a method of trapping individual cells in a microfluidic device and interrogating the cells using fluorescent imaging techniques. Appx899 (quoted above); Appx902 ("[W]e

pumped the cell suspension into the cell loading input at a flow rate of 100 µL/min . . . After the cell suspension filled the device, we stopped flow for 5 min, allowing cells to sediment into the wells."); Appx903 ("[W]e used bright-field images to define the edge of the cell and the overlaid fluorescence image to qualitatively determine fluorescence localization via user interpretation."); Appx904 ("After loading the microwell array with a 50:1 ratio of CellTracker Green/CellTracker Orange-labeled BA/F3 cells, we scanned the entire array under bright-field and fluorescence illumination and inspected the images to determine the location of orange-labeled cells."). Kovac also teaches a next step of recovering particular cells of interest by using a laser to levitate them up into a flowing fluid, which overcomes the lateral optical forces, releasing the cell and washing it downstream, where it can be collected. Appx899 (quoted above); Appx902-903 ("During removal, we used a flow rate of 2.5-5 µL/min and applied 100-150 mW of laser power to each cell until the cell was levitated high enough that the flow displaced the cell and carried it downstream."); Appx904 ("We then automatically returned to array sites containing orange-labeled cells, reinstated buffer flow, and levitated orange-labeled cells into the flow field for selective removal (Figure 4).") (emphasis added). This "selective levitation" process is illustrated in Figure 1:



Kovac describes step (c) in the figure above: "After locating cells of interest, we focus an infrared (IR) laser beam onto target cells, levitating the cells into the flow field with the optical scattering force." Appx900. The selectively levitated cells flow to a reservoir of the microfluidic device, where they are removed from the device by micropipette after the reservoir's PDMS membrane was pierced. Appx901.

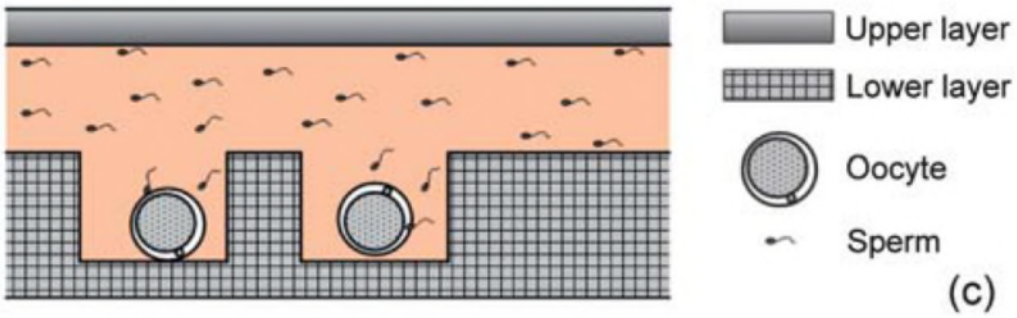
Kovac explains the decision-making behind this cell removal step, stating

> We desired to remove single cells from microwells in a manner that was straightforward, user-friendly, contact-free, and conceivably automatable. We decided that using the optical scattering force to levitate cells from microwells met these criteria and that such a system was simple to implement in a standard microscope with minimal modification.

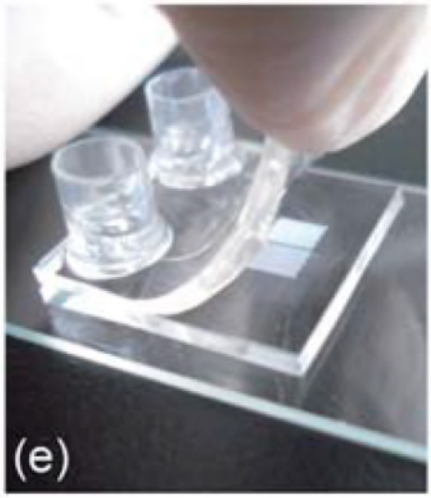
Appx903. Regarding the recovery of cells, this reference explains "[t]he technique generalizes easily to any application where the goal is to position cells in an environment, observe them using microscopy, and later retrieve particular cells." Appx907.

4. Han

Han is a journal article entitled "Integration of single oocyte trapping, *in vitro* fertilization and embryo culture in a microwell-structured microfluidic device." Appx909-915. It was published in 2010 and bears a manuscript "*Received*" date of May 5, 2010, which is before the priority date of the '408 patent (the filing date of provisional application 61/362,213). Appx909. Han's device is used for *in vitro* fertilization (IVF), and it discloses a microfluidic device that uses a microwell array to capture individual oocytes in the flow field, which is then followed by *in situ* insemination, medium changing, and embryo culture. Appx910. Like the Dimov device, the Han device "consists of two PDMS layers," and includes "a microchannel in the upper layer and a microwell array in the lower layer," as shown below. Appx910.



Han discusses the importance of being able to select healthy embryos and recover them from the microfluidic device in IVF. Appx910 (Han's system allows "track[ing] the entire fertilization and development process of any individual oocyte, providing convenience for the observation of embryo development and selection of healthy embryos for clinical use."); Appx914 ("After *in vitro* embryo culture, blastocysts need to be collected and transferred back to the uterus."). Han discloses that the top PDMS layer of the device is only partially bonded, so it could be lifted for retrieval of the cells. Appx911. Han discloses the purpose of this lifting feature: "This feature allowed us to directly remove embryos from the microwells by pipetting with the retrieval rate close to 100%." Appx914. Han includes a picture of its "microfluidic device with the upper layer lifted," shown below. Appx910.



B. Proceedings before the Board

The Board instituted *inter partes* review on one anticipation ground and four obviousness grounds, and concluded that all challenged claims were not shown to be unpatentable. In this appeal, Bruker Cellular challenges the Board's determinations with respect to the four obviousness grounds:

- Dimov in view of the knowledge of a POSA (claims 1, 6, 11, 19, 24, 26, 27, and 30)
- Dimov in view of Kovac (claims 1, 6, 11, 19, 24, 26, 27, and 30)
- Park in view of the knowledge of a POSA (claims 1, 11, 16, 24, 26, 27, and 30)
- Park in view of Kovac and the knowledge of a POSA (claims 1, 11, 16, 24, 26, 27, 30)

The Board's key underlying determinations are discussed below.

1. The Challenged Claims of the ’408 patent

Claim 1 is the only independent claim of the ’408 patent. The remainder of the challenged claims (claims 6, 11, 16, 19, 24, 26, 27, and 30) each depend from claim 1. In its Petition, Bruker Cellular demonstrated that Dimov disclosed the additional limitations of claims 6, 11, 19, 24, 26, 27, and 30, and that Park disclosed the additional limitations of claims 11, 16, 24, 26, 27, and 30. UBC did not dispute this showing and the Board based its decision on the challenged dependent claims solely on its decision on the independent claim, citing *In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988) (“Dependent claims are nonobvious under section 103 if the independent claims from which they depend are nonobvious.”). Appx34; *see also* Appx53; Appx59; Appx70. Thus, while this brief is focused on independent claim 1, the appeal extends to all challenged claims.

2. Claim Construction

In the IPR proceedings on appeal, the Board performed no express claim construction. Appx10-12. Bruker Cellular’s challenge to the claims of the ’408 patent was based on the claim construction positions taken by UBC in the *AbCellera Biologics, Inc. and the University of British Columbia v. Bruker Cellular Analysis, Inc.* litigation, which was its right, even though it disagrees with many of those claim construction positions. Appx149-152; *see, e.g., 10X Genomics, Inc. v. Bio-Rad Labs., Inc.*, IPR2020-00086, Paper 8 at 21 (PTAB April

27, 2020) (permitting the petitioner to base its challenge "on claim constructions implied by Patent Owner's district court infringement contentions without expressing subjective agreement with those constructions"). In the litigation, UBC had proposed express constructions only for the terms "chamber" and "retaining position," and the Board determined that it did not need to expressly construe these or any other terms to decide the issues in the IPR proceedings. Appx11-12.

3. Obviousness in the Final Written Decision

The Board focused its analysis on the last limitation of claim 1 ("selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step"), because there was no dispute that Dimov and Park each taught the preamble and all the other limitations of claim 1. Appx17-18; Appx55. The Board concluded that Bruker Cellular had not met its burden to show that a POSA would have been motivated to modify Dimov and Park to include the selective recovery step. Importantly, the Board expressly did not reach the issue of whether a POSA would have had a reasonable expectation of success in making any such modification. Appx51(n.15).

With respect to the obviousness grounds involving Dimov, the Board considered Bruker Cellular's contention that a POSA would have been motivated to remove a cell of interest by one of the multiple methods disclosed in the prior art: (1) by inserting a micropipette into a trench to aspirate one or more of the cells

of interest for further analysis (Appx26 (citing Appx176; Appx789(11:30-40); Appx712(¶111))); (2) using one of the removal techniques listed in Dimov, such as laser tweezers (Appx25 (citing Appx176; Appx789(11:30-40))); or (3) using Kovac's selective levitation technique to recover a cell of interest. Appx48 (citing Appx183; Appx899).

The Board's findings regarding these modifications are set forth next. The errors in the Board's reasoning and lack of substantial evidence for its findings are discussed in the argument below.

a) The Board's Findings Regarding Using Micropipette Techniques to Selectively Recover Cells

Bruker Cellular presented evidence that, based on background knowledge in the art, a POSA would have simply (1) pierced Dimov's top layer with a micropipette to remove the cell from the trench by aspiration or (2) modified Dimov's device "in a way that would permit the top layer of the device to be peeled back so a trench could be accessed directly," which would allow for such removal. Appx176-177 (citing Appx712(¶112)), Appx180 (citing Appx716(¶120)); Appx453-455 (citing Appx710-712(¶¶106-110); Appx2845-2850(¶¶69-78); Appx905-906). Because Dimov's device is "made of two layers," and Dimov does not teach bonding the two together, a POSA would have known to only partially bond the two layers to provide direct access to the trench for cell recovery. Appx177-178 (citing Appx785(3:10-12); Appx786(5:37-39);

Appx791(15:27-46); Appx783(Fig. 25); Appx713-714(¶¶113-116). This technique was also shown in Han, which reflects the use of a peelable top layer in the art before the priority date of the '408 patent. Appx404-406 (citing Appx2146-2147(¶68); Appx713(¶¶113-114); Appx714(¶117); Appx910; Appx914; Appx911). The Board found, however, that Han did not reflect the background knowledge of a POSA because Han described its device as being "novel" and distinguished its device from others that "had to generate a backward flow to make embryos return to the inlet" so they could be extracted. Appx33 (quoting Appx914). The Board did not address other evidence that POSA would have known to pierce Dimov's top layer with a micropipette to selectively recover a cell. *See, e.g.*, Appx712(¶111) (Dr. Meinhart's testimony that a POSA would have been familiar with the use of micropipettes); Appx716(¶120) (Dr. Meinhart's testimony that given "a POSA's familiarity with the use of a micropipette, a POSA would have had a reasonable expectation of success in piercing the top layer of PDMS"); Appx900 (Kovac stating that "[v]iable retrieval of small numbers of single cells from microwell arrays using micropipettes/micromanipulators based on temporal fluorescence behavior" had been demonstrated); Appx901 (Kovac disclosing piercing a PDMS membrane so a cell can be removed with a pipette).

b) The Board's Findings Regarding Using Dimov's Express Removal Techniques to Selectively Recover Cells

Bruker Cellular also presented evidence that a POSA conducting experiments with Dimov's device and observing an interesting cell, would have been motivated to use one of the techniques expressly disclosed in Dimov to selectively remove that cell from a trench for further investigation. Appx175-176. Dimov identified techniques to "effect removal of the particles out of the trench," and lists the following ways to do this:

> Acoustic
> Magnetic
> Inertial
> Electric
> Dielectrophoretic
> Thermo-hydrodynamic
> Laser tweezers
> Hydrodynamically induced agitation
> Specific or unspecific attachment to surface.

Appx789(11:26-40) (quoted by Appx167). Dimov expressly teaches that these "particles" can be cells. Appx787(7:4-5, 7:8-9); *see also id.* at Appx784(1:57) (highlighting that "the particles are cells" in an embodiment). Dimov further teaches that the use of such techniques may require an external source of agitation or manipulation of the particles. Appx167 (citing Appx789(11:41-43); Appx702(¶87)). Dimov's "common output" and common waste" features provide convenient outlets for accessing cells, and Bruker Cellular presented evidence that

a POSA would have known how to use Dimov's identified techniques (*e.g.*, laser tweezers) with microfluidic devices. Appx450-452 (citing Appx2815(¶¶11-12); Appx2825-2836(¶¶31-48); Appx2840-2845(¶¶60-68).

The Board disagreed, finding that Dimov's reference to "removal of particles from a trench would have been insufficient to teach a [POSA] to effectively recover a cell if their motivation [were] to conduct further investigations on the cells by, for example, sequencing or culturing." Appx29. The Board credited the testimony of UCB expert Dr. Gale that a POSA "may *at least* as plausibly have interpreted" Dimov's disclosure of "effect[ing] removal of the particles out of the trench" using different techniques as being limited to "removing waste or other unwanted particles out of the capture chamber/trench" (Appx30), despite Dimov expressly teaching that these "particles" can be cells. Appx787(7:4-5, 7:8-9); *see also* Appx784(1:57). The Board faulted Dimov for "merely list[ing] a set of techniques and provid[ing] no guidance on how to carry out such removal in a way that would still allow for further meaningful analysis of cells." Appx30 (citing Appx789(11:21-43)). The Board further found that Dimov "also does not address the difficulties a person of ordinary skill in the art would face in finding and recovering a cell that has been ejected into Dimov's common waste stream." Appx31.

c) The Board's Findings Regarding Using Kovac's Selective Levitation Technique to Selectively Recover Cells

With respect to the Kovac modification, Bruker Cellular presented evidence that, based on Kovac's teachings, a POSA would have been motivated to use a focused infrared laser to levitate cells of interest from Dimov's trenches, so those cells would flow out of the trench and could be recovered. Appx180-185. Kovac identified an "ubiquitous" need to "isolate particularly interesting cells for further investigation." Appx899. Kovac teaches meeting this need through its "selective levitation" technique, which could selectively recover a single cell from a microfluidic device. Appx900(Fig. 1(a) caption) ("After locating cells of interest, we focus an infrared (IR) laser beam onto target cells, levitating the cells into the flow field with the optical scattering force."); Appx904 ("A single cell can, in general, even be removed selectively from a well with multiple cells (Figure 3B, Supporting Information movie S-2), a testament to the tight localization of the optical force."); *see also* Appx717-718(¶¶124-125). Kovac further teaches that this technique "was straightforward, user-friendly, and conceivably automatable" and "was simple to implement in a standard microscope with minimal modification." Appx903; *see also* Appx718-719(¶126).

The Board concluded, however, that Bruker Cellular had "failed to meet its burden to show that a person of ordinary skill in the art would have selected

Dimov's microfluidic device for use with Kovac's cell levitation method." Appx49. The Board's first basis for its decision was finding that Kovac's specific technique was used to levitate cells out of a "microwell that is nearly an order or magnitude narrower and shallower than Dimov's trench" and that there were many "factors at play" in obtaining laser parameters (*e.g.*, power, spot size, and exposure time) for raising a cell out of Dimov's trench. Appx50-51. The Board also found that there was a likelihood of unacceptable cell damage under certain combinations of laser power, spot size, and exposure time. Appx50. Based on these findings, the Board decided that Bruker Cellular had not shown that "a person of ordinary skill in the art would have had the background knowledge and experience needed for manipulating the many 'factors at play' to successfully adapt Kovac's method for use in Dimov's much deeper trenches." Appx51.

The Board's second basis for its decision was that it had not been shown that a POSA "would have considered it feasible to recover liberated cells from Dimov's shared waste stream, given the long path from each trench to the waste stream and the fact that potentially hundreds of other trenches share the same outlet." Appx 52 (citing Appx19-20, Appx24).

4. The Request for Rehearing

Bruker Cellular timely filed a request for rehearing of the Board's final written decision that claims 1, 6, 11, 16, 19, 24, 26, 27, and 30 were not shown to

be unpatentable by a preponderance of the evidence according to Grounds 2-5. Appx638. Bruker Cellular pointed out that the Board had misapprehended or overlooked its arguments and evidence in that the Board:

(1) committed legal error by erroneously basing its motivation-to-combine decision on reasonable-expectation-of-success evidence. (Appx648.)

(2) committed legal error by looking to Dimov and Park alone for motivation to combine and overlooked evidence of the contributions of POSA's knowledge. (Appx639-641; Appx649-650.)

(3) overlooked substantial evidence that a POSA would have been motivated to, and had a reasonable expectation of success in, modifying Dimov and Park to use a variety of techniques (including that taught by Kovac) to selectively recover a cell of interest. (Appx641-643; Appx643-647.)

(4) committed legal error by disregarding Han's evidence of the knowledge of a POSA. (Appx643.)

The Board denied the request for rehearing. Appx73-88. This appeal followed.

V. SUMMARY OF THE ARGUMENT

For four reasons, this Court should reverse or at a minimum vacate the Board's decision on appeal.

First, the Board committed legal error when it disregarded fundamental motivation-to-combine principles by basing its decision on purported difficulties in

modifying Dimov and Park. Whether a POSA *would have been motivated* to modify Dimov and Park to selectively recover a cell from those particular microfluidic devices is a completely different question—based on different considerations and broader evidence—from whether a POSA *would have a reasonable expectation of success in modifying* either reference's device. Motivation to combine and reasonable expectation of success are distinct requirements in an obviousness analysis. By basing its motivation-to-combine decision on reasonable-expectation-of-success evidence, the Board misjudged whether a POSA would have been motivated to achieve the claimed invention.

Second, the Board committed legal error when it required the motivation to modify the Dimov and Park references to be found in the references themselves. The motivation to modify a reference need not be found in the reference itself; it could come from another reference or a POSA's background knowledge. In an obviousness analysis, where a reference is missing a claim limitation, it is tautological error to fault the reference for failing to provide motivation by teaching or suggesting the missing limitation.

Third, there was no substantial evidence to support the Board's finding that there was no motivation to modify Dimov and Park to include selective cell recovery. Claim 1 is a method claim, and there is no dispute both Dimov and Park teach retaining a cell in a chamber of a microfluidic device, perfusing it by flowing

fluid through the chamber (which has an inlet and a suitably positioned outlet), culturing the cell within the chamber, and monitoring a response in the chamber. The last step of claim 1, "selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step," is something that any POSA would have been motivated to do. For example, Kovac described an "ubiquitous" need to "isolate particularly interesting cells for further investigation." Appx899. POSAs were already motivated to obtain "interesting" cells that had been identified by their microfluidic experiments, and so would have been motivated to use particular selective cell recovery techniques. Such techniques included the standard lab practice of pipetting (as known in the art, taught by Kovac, and disclosed by Han), various cell-manipulation techniques (as taught by Dimov), and laser levitation (as taught by Kovac). Additionally, the Board committed legal error when it found Han did not reflect the background knowledge of a POSA when evaluating motivation to combine.

VI. ARGUMENT

A. Standard of review

"Obviousness is a question of law based on subsidiary findings of fact." *In re Van Os*, 844 F.3d 1359, 1360 (Fed. Cir. 2017). The Court reviews "the Board's legal determination of obviousness de novo and its factual findings for substantial evidence." *Outdry Techs. Corp. v. Geox S.P.A.*, 859 F.3d 1364, 1367 (Fed. Cir.

2017). "The Board's motivation to combine finding is reviewed for substantial evidence," and "[t]he Board must support its finding . . . with a reasoned explanation to enable . . . review for substantial evidence." *Id.* at 1368.

B. The Board committed reversible error by focusing on reasonable-expectation-of-success evidence when deciding whether there was a motivation to combine.

"A determination of obviousness requires finding that a person of ordinary skill in the art would have been motivated to combine or modify the teachings in the prior art and would have had a reasonable expectation of success in doing so." *Adapt Pharma Operations Ltd. v. Teva Pharms. USA, Inc.*, 25 F.4th 1354, 1365 (Fed. Cir. 2022) (citation and quotation marks omitted). Whether a POSA would have been motivated to improve a particular prior art device or method is a ***separate*** question from whether a POSA would reasonably expect to achieve success in making the improvement. This Court has clearly and emphatically explained this distinction:

> [W]e must first emphasize the clear distinction in our case law between a patent challenger's burden to prove that a skilled artisan would have been motivated to combine prior art references and the additional requirement that the patent challenger also prove that the skilled artisan would have had a reasonable expectation of successfully achieving the claimed invention from the combination.

Eli Lilly & Co. v. Teva Pharms. Int'l GmbH, 8 F.4th 1331, 1344 (Fed. Cir. 2021). "Motivation to combine" and "reasonable expectation of success" are "two different legal concepts." *Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd.*,

821 F.3d 1359, 1367 (Fed. Cir. 2016) ("The reasonable expectation of success requirement refers to the likelihood of success in combining references to meet the limitations of the claimed invention."). Thus, evidence that it might be difficult to modify a particular device or method with a particular known improvement may be evidence that there is no reasonable expectation of success, but it is not relevant evidence that a POSA would not have been motivated to make the improvement.

Because the Board cited *Intelligent Bio-Systems* in its Final Written Decision (Appx51-52(n.15)) when confirming it was only making motivation-to-combine findings and not any reasonable-expectation-of-success findings, a discussion of the case would be helpful. The Board claimed that *Intelligent Bio-Systems* stood for the proposition that "although reasonable expectation of success considers the invention as claimed, a skilled artisan's ability to achieve the intended outcome is relevant to the motivation to combine." Appx51-52(n.15) (citing Intelligent Bio-Systems, 21 F.3d at 1367-68). That characterization reads too much into the case, which was about the effect of ***operability*** on motivation to combine, not the level of skill of a POSA. In *Intelligent Bio-Systems*, which involved chemical reactions used for polynucleotide sequencing by synthesis (SBS), "the petitioner's ***sole*** argument for why one of skill in the art would be motivated to combine Zabgorodny's azidomethyl group with Tsien's SBS method was because it would meet Tsien's quantitative deblocking requirement." 21 F.3d at 1368 (emphasis

added). But the evidence was that Zabgorodny's azidomethyl group would ***not*** have met Tsien's requirement, nor would a POSA have reason to believe that it would. *Id.* at 1368-69. That was because Tsien's requirement was near-100% reaction efficiency (*id.* at 1364), whereas the prior art taught that the efficiency when using an azidomethyl group would be 60-80% or less. *Id.* at 1368-69. Thus, the Board read too much into *Intelligent Bio-Systems* in defense of its faulty analysis.

The Board here erred by conflating distinct concepts and failing to evaluate motivation-to-combine evidence separate from evidence that went to reasonable expectation of success. When motivation to combine is evaluated properly, there is not substantial evidence supporting the Board's finding that there was no motivation to combine, for the reasons discussed below. The Court should reverse that finding; at a minimum, remand is required.

Here, it is undisputed that two primary references—Dimov and Park—each teach all the steps of claim 1 except the last: "selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step." Appx18; Appx55. There was ample evidence before the Board that a POSA, after using ***any*** microfluidic device to run experiments that show one or more cells respond differently than others, would have been motivated to recover these particularly interesting cells for further investigation (*e.g.*, by

sequencing or culturing them). Appx176; Appx712(¶110); *see infra* at 40-47. Such "motivation to combine may be found explicitly or implicitly in market forces; design incentives; the interrelated teachings of multiple patents; any need or problem known in the field of endeavor at the time of invention and addressed by the patent; and the background knowledge, creativity, and common sense of the person of ordinary skill." *Adapt Pharma*, 25 F.4th at 1365 (quotation omitted). Here, Bruker Cellular demonstrated that selectively recovering cells of interest was a known need in the art—one written about (and actually performed) by skilled artisans and admitted to by the '408 patent applicants. And it is only common sense that a POSA would be motivated to recover a particular cell their microfluidic experiments had shown had interesting properties or behaviors. Indeed, patentee UBC should have a difficult time arguing anything contrary, having written in the applications leading to the '408 patent that: "[c]ell recovery is often required to enable functional assays to be performed on the progeny of the input cells, or to select cells of interest for larger scale culture. A method to recover defined clonal populations is therefore a critical requirement for many applications of microfluidic cultures." Appx125(26:1-6); Appx1350(40:5-8); *see also* Appx899 (Kovac describing the "ubiquitous" need to "isolate particularly interesting cells for further investigation").

Thus, if a POSA had known of a particular techniques available for selectively recovering a cell from a microfluidic chamber, they would have been motivated to apply those known techniques to their microfluidic experiments, and thereby achieve claim 1 of the '408 patent. As a result, claim 1 would have been obvious, unless the actual application of the technique to a particular device was beyond a POSA's skill, in which case the POSA would not have a reasonable expectation of success. *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 417 (2007) ("[I]f a technique has been used to improve one device, and a person of ordinary skill in the art would recognize that it would improve similar devices in the same way, using the technique is obvious unless its actual application is beyond his or her skill.").

And therein lies a significant legal error committed by the Board below. The Board rendered its decision entirely on whether Bruker Cellular had met its burden to show motivation to combine, but it did not consider all of the evidence. Once it came to the motivation-to-combine conclusion, the Board expressly did not reach the issue of reasonable expectation of success. Appx51(n.15).

Yet in finding there was no motivation to combine, the Board relied on evidence regarding whether a POSA would have had the skill to implement various cell-recovery techniques in the Dimov and Park devices. These examples from the

Board's final written decision illustrate the Board's legal error in confusing motivation to combine with reasonable expectation of success:

> Even if Dimov does suggest removing cells to conduct further analyses outside the device, the reference merely lists a set of techniques and provides no guidance on ***how to carry out*** such removal in a way that would still allow for further meaningful analysis of cells. (Appx30 (citing Appx789(11:21-43)) (emphasis added).)
>
> Dimov also does not address the ***difficulties*** a person of ordinary skill in the art would face in finding and recovering a cell that has been ejected into Dimov's common waste stream." (Appx31 (emphasis added).)
>
> We also find that Petitioner has not shown that a person of ordinary skill in the art would have considered it ***feasible*** to recover liberated cells from Dimov's shared waste stream . . . . (Appx52 (emphasis added).)
>
> Petitioner has not shown that such a person would have had sufficient background in using lasers to manipulate living cells that they ***could have adapted*** Kovac's levitation method for use in Park's device without additional inventive steps. (Appx68 (emphasis added).)

In each example, the Board has focused on whether a POSA would likely have expected to succeed in making the modification (reasonable expectation of success), and not on whether a POSA would have thought or desired to make the modification (motivation to combine). Further examples are discussed below in connection with the Board's lack of substantial evidence for its finding of no motivation to combine.

When Bruker Cellular challenged the Board's legal reasoning it its petition for rehearing, the Board repeated its flawed logic, looking to reasonable-expectation-of-success evidence in reaching a motivation-to-combine conclusion:

> In determining whether there would have been a motivation to combine Dimov with Kovac, we must consider "the background knowledge possessed by a person having ordinary skill in the art" and "the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 418 (2007). In light of these considerations, we found that Petitioner failed to show that a person of ordinary skill in the art (as Petitioner articulated it) ***would have had the background necessary to adapt*** Kovac's laser parameters for use in Dimov's device ***to successfully recover*** usable cells from Dimov's output or waste streams.

Appx80-81 (emphasis added). Whether a POSA had the ***skill*** to make a modification successfully is different from whether a POSA would have been motivated to do so. *KSR*, 550 U.S. at 417 (holding it would be obvious for a POSA to use a known technique to make an improvement "unless its actual application is beyond his or her skill"). By basing its motivation-to-combine decision on evidence going instead to the reasonable-expectation-of-success question, while at the same time not reaching that question, the Board misjudged whether a POSA would have been motivated to achieve the claimed invention.

Under a proper legal standard for considering a POSA's motivation, there is no substantial evidence for the Board's finding that there was no such motivation in this case, as discussed below. Thus, the Board's decision cannot be affirmed.

C. The Board committed legal error when it required the Dimov and Park references to motivate their own modification.

The Board committed a second legal error, by requiring the motivation to modify Dimov and Park to come from the references themselves. The Board did initially accurately summarize Bruker Cellular's argument as to Dimov:

> According to Petitioner . . . persons of ordinary skill in the art would have been motivated to selectively remove cells based on the monitored response [in Dimov], as recited in limitation 1E, so that they could further investigate cells of interest cultured in the chamber (such as to sequence their DNA or to expand the cells by culturing).

Appx25 (citing Appx175-176; Appx711-712(¶¶107-110)). As to the Park reference, the Board also correctly summarized Bruker Cellular's argument: "It would have been obvious to a [person of ordinary skill in the art] to make use of this monitoring [in Park] by selectively removing one or more cells from an individual microwell based on a response—*e.g.*, a long-term cell response or an instantaneous cell reaction—observed through such monitoring.") Appx56 (citing Appx197; Appx734-735(¶160)).

The Board did not, however, account for the evidence supporting those arguments showing that the background knowledge of a POSA had already motivated them to selectively recover cells they found interesting. *See infra* at 40-47. Instead, the Board focused on whether Dimov and Park by themselves motivated their own modification:

> In light of [Dimov's] design, Petitioner has not persuasively shown that a person of ordinary skill in the art ***would have inferred, from Dimov's brief reference*** to "removal of the particles out of the trench," ***a teaching to conduct further analyses outside of the device*** (such as culturing or sequencing) on cells determined to be of interest based on a prior analysis. (Appx30 (emphasis added).)
>
> ***Nor is Dimov's mention of laser tweezers sufficient to motivate*** a person of ordinary skill in the art to remove cells from a trench for further analysis outside the device. (Appx30-31 (emphasis added).)
>
> We agree with Patent Owner that Petitioner has not pointed to anything in Park that teaches selectively recovering cells. Petitioner's attempt to infer such a motivation from Park's disclosure relies on improper hindsight bias***. In particular, Park itself does not appear to motivate*** making the top layer removable and aspirating cells from microwells using a micropipette. (Appx58 (emphasis added) (citation omitted).)

But motivation to combine need not be found in the primary references, it "may be found explicitly or implicitly in market forces; design incentives; the interrelated teachings of multiple patents; any need or problem known in the field of endeavor at the time of invention and addressed by the patent; and the background knowledge, creativity, and common sense of the person of ordinary skill." *Adapt Pharma*, 25 F.4th at 1365 (quotation omitted); *see also Nat'l Steel Car, Ltd. v. Canadian Pac. Ry.*, 357 F.3d 1319, 1337 (Fed. Cir. 2004).

By focusing on Dimov's and Park's teachings to the exclusion of the knowledge of a POSA, the Board committed legal error. This caused the Board to ignore the substantial evidence that a POSA would have the knowledge and common sense to be motivated to recover a cell of interest from ***any*** microfluidic

device, including Dimov's and Park's, regardless of those two references' specific teachings. Appx169-170; Appx176; Appx451; Appx705-706(¶¶92-93); Appx711-712(¶¶108-110); Appx717(¶122); Appx718-719(¶126); Appx734-735(¶160); Appx2841(¶ 61); Appx2850-2851(¶¶80-81); Appx125(26:1-6); Appx1350(40:5-6).

When Bruker Cellular challenged the Board's focus on Dimov and Park to the exclusion of other evidence in its request for rehearing, the Board claimed that it did not misapprehend Bruker Cellular's arguments. Appx640. But the Board's characterization of its Final Written Decision shows that the Board did not consider the background knowledge in the art ***except*** as it related to the reasonable-expectation-of-success evidence, which, as discussed above, is a separate and distinct inquiry:

> In the Decision, we did not find Petitioner's arguments persuasive in light of the background knowledge in the art. In particular, we found that Petitioner had not adequately shown that ***the background knowledge in the art*** would have been sufficient to inform a person of ordinary skill in the art ***how to apply*** the techniques such as "laser tweezers" that Dimov mentions briefly, in a way that would have allowed further analyses of cells outside Dimov's device.

Appx76 (citing Appx29-31) (emphasis added). Knowing "how to apply" techniques to achieve an intended goal speaks only to reasonable expectation of success. But the POSA's "background knowledge in the art" in this case also speaks to motivation—it included the recognition that the "need to isolate small numbers of specific cells from background populations is ubiquitous, with

applications in pathology, clinical diagnosis, cloning, and cell biology research." Appx899 (cited in Appx181). The Board's failure to address the ubiquity of this need when assessing motivation, and myopic focus on the primary references for motivation instead, was legal error requiring reversal or, at a minimum, remand.

D. There was no substantial evidence to support the Board's finding that there was no motivation to modify Dimov and Park to include selective cell recovery.

The last step of claim 1 is "selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step." The need for performing this step—and thus the motivation for modifying any microfluidic cell-experiment method to include it—was already known in the art and to a POSA. For example, Kovac expressly states this need is ubiquitous:

> The need to isolate small numbers of specific cells from background populations ***is ubiquitous***, with applications in pathology, clinical diagnosis, cloning, and cell biology research. In the context of cell biology experiments, sorting can be a way to select a desired starting population of cells of known characteristics or ***can be a tool to analyze the results of an experiment and isolate particularly interesting cells for further investigation***.

Appx899 (emphasis added); Appx181. The '408 patent applicants expressed the same thought when they wrote in their provisional application that

> Cell recovery is ***often*** required to enable functional assays to be performed on the progeny of the input cells, or to select cells of interest for larger scale culture. ***A method to recover defined***

> ***clonal populations is therefore a critical requirement for many applications of microfluidic cultures.***

Appx125(26:1-6) (emphasis added); Appx1350(40:5-8); *see also* Appx2850-2851(¶¶80-81). The applicants' admission that cell recovery is "often" required is a restatement of the known, "ubiquitous" need. Appx456. Such "applicant admitted prior art" may furnish a motivation to combine. *Qualcomm Inc. v. Apple Inc.*, 24 F.4th 1367, 1369, 1376 (Fed. Cir. 2022); *see also* Appx457. The evidence also showed that a POSA would know there are many situations where one would want to selectively recover cells. Appx3422-3423(31:16-32:23); Appx3424(33:7-17); Appx3428-3429(37:24-38:12). Indeed, "any need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed." *KSR*, 550 U.S. at 420. After monitoring cellular responses in a microfluidic device to identify cells of interest, recovering those cells from the device for further analysis was a known need—and thus a reason for modifying Dimov and Park to do so.

Needing to recover a cell of interest, a POSA would then be motivated to turn to methods of selective cell recovery and apply them to Dimov's and Park's devices. Three categories of such methods were before the Board: pipetting techniques (Appx176; Appx789(11:30-40); Appx712(¶111)); the removal techniques listed in Dimov, such as laser tweezers (Appx176; Appx789(11:30-

40)); and Kovac's selective levitation technique (Appx183; Appx899). These are addressed in turn.

1. A POSA would have been motivated to use known selective cell-recovery pipetting techniques.

A POSA would have known of various techniques of using a micropipette to obtain particular cells from a microfluidic device, which was a standard practice in the art. For example, Kovac states that "[v]iable retrieval of small numbers of single cells from microwell arrays using micropipettes/micromanipulators based on temporal fluorescence behavior has [] been demonstrated," citing to articles published in 2004 and 2005. Appx900; *see* Appx181; Appx185; Appx717(¶122); *see also* Appx905-906 ("Cell retrieval from the device requires a simple pipetting step, which is ***standard lab practice*** and unlikely to damage cells." (emphasis added)). A POSA would have known that a micropipette could access a cell of interest in a chamber (*e.g.*, Dimov's trench or Park's well) by piercing the device's top PDMS layer with the micropipette. Appx180 (citing Appx716(¶120)); Appx176-177 (citing Appx712(¶111); Appx783(Fig. 25); Appx785(3:10-12); Appx786(5:37-39); Appx791(15:27-46)); Appx454 (citing Appx2845-2850(¶¶69-78); see also Appx2650(39:7-22) (UBC's expert Dr. Gale admitting "there were times before 2010 where cells were moved around using micropipettes.") (cited in Appx2835-2836(¶48); Appx451). Kovac discloses that a PDMS membrane can be pierced so that a cell can be removed with a pipette. Appx901; Appx901(Fig. 2).

This teaching was acknowledged by the Board. Appx38. In light of pipetting being a standard lab practice, the Board's conclusion that a POSA would not have known or been motivated to pierce a PDMS layer to pipette a cell out of Dimov's or Park's device lacks substantial evidentiary support. *KSR*, 550 U.S. at 421 ("A person of ordinary skill is also a person of ordinary creativity, not an automaton.").

A POSA would also have known that a micropipette could access a cell of interest in a chamber by peeling back the device's top PDMS layer to expose the chamber. Dimov discloses a two-layer device (having a fluidic layer and a lid/intel layer) fabricated by "bringing the first and second layers together and assembling them relative to one another onto a glass substrate," but does not disclose that the layers are bonded together. Appx791(15:27-46). A POSA would know that unbonded (or even partially bonded) layers in Dimov would permit micropipette access to the trenches by peeling back the top (lid) layer. Appx713(¶¶113-114); Appx177-178. Indeed, Han discloses two partially bonded PDMS layers that "allowed us to directly remove embryos from the microwells by pipetting with the retrieval rate close to 100%." Appx914; Appx911 ("The microchamber region was left unbonded so that the upper layer can be partially lifted for the retrieval of blastocysts."); Appx180-181. Despite this teaching, the Board concluded Han did not reflect the background knowledge of a POSA because "Han characterized its microfluidic device as 'novel' and distinguished it from previous devices known in

the art." Appx79. That conclusion was legal error. In *National Steel Car, Ltd. v. Canadian Pacific Railway*, the Court considered whether a drawing that had not been disseminated (and so was not prior art), nevertheless reflected the knowledge of a POSA such that it was "relevant to whether a motivation to combine . . . is implicit in the knowledge of one of ordinary skill in the art." 357 F.3d 1319, 1337-38 (Fed. Cir. 2004). The Court concluded that it was relevant, because the inquiry was whether the drawing demonstrated that others in the drawer's position would have considered it obvious to combine the elements found in the prior art. *Id.* at 1338. Here, Han is relevant evidence of a motivation to modify Dimov and Park with a peelable layer because it demonstrates that others in the position of the Han authors would have had the same motivation. And whether Han is prior art is not relevant, because post-priority-date publications that report on pre-priority-date work can be evidence of a POSA's motivation. *Yeda Research v. Mylan Pharm. Inc.*, 906 F.3d 1031, 1041 (Fed. Cir. 2018)).

A POSA, who would have been motivated to selectively recover cells so they could further investigate cells of interest, would likewise have been motivated to use the standard lab practice of pipetting to selectively recover a cell of interest from Dimov's and Park's devices, whether directly from the chamber or from an output (had the cell been removed from the chamber so it could flow to the output).

2. A POSA would have been motivated to use Dimov's selective cell removal techniques.

Dimov discloses a microfluidic device in which "particles provided within a fluid flowing within the fluid path will preferentially collect within the capture chamber," and that "[t]he capture or collection chamber is desirably in the form of a trench[.]" Appx784(1:46-47); Appx784(1:52-53). Dimov teaches these "particles" can be cells. Appx787(7:4-5, 7:8-9); *see also id.* at Appx784(1:57) (highlighting that "the particles are cells" in an embodiment). Dimov teaches that "it is possible to modify the arrangement so as to provide for subsequent movement of the particles—either within the trench so as to provide for mixing or the like, ***or to effect removal of the particles out of the trench***." Appx789(11:23-26) (emphasis added). "Such arrangements will typically require a capacity to manipulate the particles and this can be conducted either before or subsequent to capture of the particles within the trench." Appx789(11:27-30). Dimov identified a variety of techniques for removing a cell:

> Examples of techniques that could be employed include:
> Acoustic
> Magnetic
> Inertial
> Electric
> Dielectrophoretic
> Thermo-hydrodynamic
> Laser tweezers
> Hydrodynamically induced agitation
> Specific or unspecific attachment to surface.

Appx789(11:30-40). A POSA would have recognized that after removing a cell of interest from "capture chamber 160" (or trench) in the Dimov device, the cell would then flow down "a fluid path 103 defined within a substrate 105" to "an output 130," or possibly even further to the structure labeled "common waste 140," where it could then be removed from the device by micropipette, as Dimov's "common output" or "common waste" are convenient outlets for accessing cells. Appx184; Appx451; Appx720-721(¶129); Appx785(3:22-26); Appx785(4:21-22); Appx763(Fig. 1); Appx764(Fig. 2); Appx2825-2836(¶¶31-48).

As with the motivation to use Kovac's selective recovery technique, there was no substantial evidence for the Board's finding that a POSA would not have been motivated to use Dimov's selective removal techniques. Here, too, the Board confused reasonable expectation of success for motivation:

> we found that Petitioner had not adequately shown that the background knowledge in the art would have been sufficient to inform a person of ordinary skill in the art ***how to apply*** the techniques such as "laser tweezers" that Dimov mentions briefly, in a way that would have allowed further analyses of cells outside Dimov's device.

Appx641 (citing Appx29-31) (emphasis added). The Board continued that confusion, adding the legally erroneous requirement that Dimov itself provide the necessary teachings:

> [W]e found that Petitioner had not shown that Dimov's teaching that the device can be modified to allow removal of particles from a trench through techniques such as "laser tweezers" ***was sufficient***

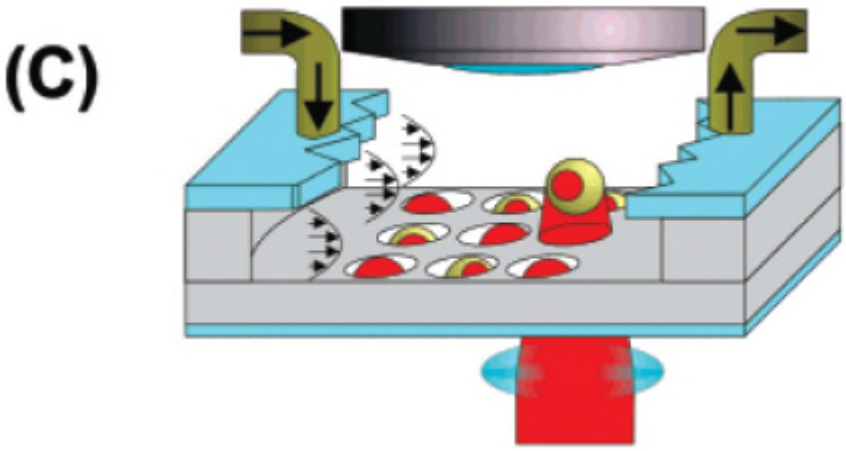
> ***to teach selective recovery*** of cells suitable for use in further investigations such as sequencing or culturing. *See* [Appx29-31]; *see also* [Appx24] (crediting Dr. Gale's testimony that there would have been substantial obstacles to recovering specific cells liberated from Dimov's trenches). Even if Dimov were read to include a teaching in that regard, we found that Petitioner's argument was missing any persuasive showing that the ***specifics of conducting such removal*** would have been within the ordinary level of skill and creativity in the art at the time of the claimed invention.

Appx641 (citing Appx29-31) (emphasis added). The "ubiquitous" need to selectively recover cells suitable for use in further investigations such as sequencing or culturing was well-known in the art, and the specifics of successfully conducting such removal concern reasonable expectation of success, not motivation. There was not substantial evidence for the Board's finding that a POSA would not have been motivated to use Dimov's removal techniques to selectively recover cells of interest.

3. A POSA would have been motivated to use Kovac's selective cell-recovery technique using laser levitation.

A POSA would have been motivated to use Kovac's selective cell-recovery technique using Dimov's and Park's devices. Dimov teaches various ways in which cells could be selectively recovered, including "laser tweezers." Appx789(11:27-40). Kovac discloses a particular implementation of Dimov's "laser tweezers" technique that a POSA would have been motivated to use. Specifically, Kovac teaches "us[ing] the scattering force from a focused infrared laser to levitate cells of interest from their wells into a flow field for collection"

from a microfluidic device, thereby "combining the steps of physical selection and target removal into a single step." Appx899; Appx903; Appx900 ("Here we present a microscope-compatible, array-based microfluidic cell sorting architecture centered around passive hydrodynamic trapping of cells and active release using the optical scattering force (Figure 1)."). Appx717-718(¶124). Kovac refers to this technique as "selective levitation," Appx903, illustrated in Figure 1(c) below.



Appx900; Appx181-182. The selectively levitated cells flow to a reservoir of the microfluidic device, where they are removed from the device by micropipette. Appx901 (explaining that after "target cells flow into the reservoir, we use a standard 200 µL pipettor to transfer cells" out of the microfluidic device); *Id.* (Fig. 2); Appx718(¶125); Appx182. Kovac teaches this technique "was straightforward, user-friendly, and conceivably automatable," and "was simple to implement in a standard microscope with minimal modification," Appx903, "requir[ing] only flow and a clear optical path to the chip." Appx900; Appx183.

A POSA, who would have been motivated to selectively recover cells so they could further investigate cells of interest, would likewise have been motivated to use Kovac's laser levitation technique to do so. A POSA would have known, as Kovac teaches, that this technique provides "user-friendly, image-based selection and removal of particular target cells from a background population" in a way that "combines the simplicity and wide-area advantages of microwell arrays with the intuitive operation of optical techniques." Appx900.

There was no substantial evidence for the Board's finding that a POSA would not have been motivated to use Kovac's selective recovery technique in Dimov's device. Again, the Board focused on the expectation of success of the combination, instead of the motivation to combine. The Board's summary of its findings in its rehearing denial is: "In light of these considerations, we found that Petitioner failed to show that a person of ordinary skill in the art (as Petitioner articulated it) would have had the background necessary to adapt Kovac's laser parameters for use in Dimov's device to ***successfully*** recover usable cells from Dimov's output or waste streams." Appx464 (citing Appx50-52) (emphasis added). All the considerations weighed by the Board spoke to whether a POSA would have a reasonable expectation of success, not whether a POSA would be motivated to make the modification. This is reversible error. *Eli Lilly*, 8 F.4th at 1344 (holding there is a "clear distinction" between motivation to combine and a

reasonable expectation of success). Kovac demonstrated successful, selective recovery of cells from microwells while maintaining cell viability (Appx899), and that would have motivated a POSA to use the same technique with Dimov and Park, particularly given Kovac's teaching that its "technique generalizes easily to any application where the goal is to position cells in an environment, observe them using microscopy, and later retrieve particular cells." Appx907.

VII. CONCLUSION AND STATEMENT OF RELIEF SOUGHT

The Board's decision should be reversed. When the Board's legal errors are corrected, and its factual determinations that lack substantial evidence are set aside, the record permits only one conclusion: each of Dimov and Park, when supplemented with the knowledge of a POSA or with the teachings of Kovac, render obvious the claimed inventions.

At a minimum, the case should be remanded for the Board to correct its obviousness analysis by applying the correct legal standards for evaluating motivation to combine to the evidence.

Dated: December 1, 2023

Respectfully submitted,

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ADDENDUM

ADDENDUM TABLE OF CONTENTS

Trials@uspto.gov
571-272-7822

Paper 38
Entered: January 19, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BERKELEY LIGHTS, INC.,
Petitioner,

v.

THE UNIVERSITY OF BRITISH COLUMBIA,
Patent Owner.

IPR2021-01249
Patent 10,087,408 B2

Before KRISTINA M. KALAN, CHRISTOPHER M. KAISER, and CHRISTOPHER L. OGDEN, *Administrative Patent Judges*.

OGDEN, *Administrative Patent Judge*.

JUDGMENT
Final Written Decision
Determining No Challenged Claims Unpatentable
35 U.S.C. § 318(a)

I. INTRODUCTION

Berkeley Lights, Inc. ("Petitioner") filed a Petition (Paper 1, "Pet.") for *inter partes* review of claims 1, 6, 11, 16, 19, 24, 26, 27, and 30 of U.S. Patent No. 10,087,408 B2 (Ex. 1001, "the '408 patent"). The University of British Columbia ("Patent Owner") filed a Patent Owner Response (Paper 18, "PO Resp."), Petitioner filed a Reply to the Patent Owner Response (Paper 22, "Pet. Reply"), and Patent Owner filed a Sur-reply (Paper 29, "PO Sur-reply").

We held an oral hearing on November 4, 2022, and the transcript is entered on the record. Paper 37 ("Tr.").

This is a final written decision under 35 U.S.C. § 318(a) as to whether the claims challenged in the *inter partes* review are unpatentable. For the reasons below, we conclude that Petitioner has not shown that any claims of the '408 patent are unpatentable.

II. BACKGROUND

A. RELATED PROCEEDINGS

As related matters, the parties identify the following three pending U.S. district court cases: *AbCellera Biologics, Inc. v. Berkeley Lights, Inc.*, No. 5:20-cv-08627 (N.D. Cal. filed July 9, 2020); *AbCellera Biologics, Inc. v. Berkeley Lights, Inc.*, No. 5:20-cv-08626 (N.D. Cal. filed Aug. 25, 2020); *AbCellera Biologics, Inc. v. Berkeley Lights, Inc.*, No. 5:20-cv-08624 (N.D. Cal. filed Sept. 16, 2020) (collectively, "parallel district court proceedings"). Pet. 3–4; Paper 6, 1. The District Court has stayed these proceedings

pending the outcome of this and two other *inter partes* review proceedings. *See* Ex. 1035.

We note that *inter partes* reviews IPR2021-01250 (institution denied on January 21, 2022) and IPR2021-01386 (institution denied on February 1, 2022) also involved the same parties and related technology.

B. THE '408 PATENT (EX. 1001)

The '408 patent issued from an application filed July 7, 2011, claiming priority from U.S. Provisional Application No. 61/362,213, filed July 7, 2010. Ex. 1001, codes (22), (60). It describes methods, in the context of microfluidic devices, "for perfusing a cell with perfusion fluid," so that "the gravitational forces acting on the cell to keep the cell at or near . . . a retaining position exceed the hydrodynamic forces acting on the cell to move it toward an outlet." *Id.* at code (57).

Figure 1 of the '408 patent, reproduced below, is a top view of the microfluidic device, with two expanded views at medium resolution (top left, with a 1 mm size reference bar) and high resolution (top right, with a 100 μm size reference bar). Ex. 1001, 6:17–21.

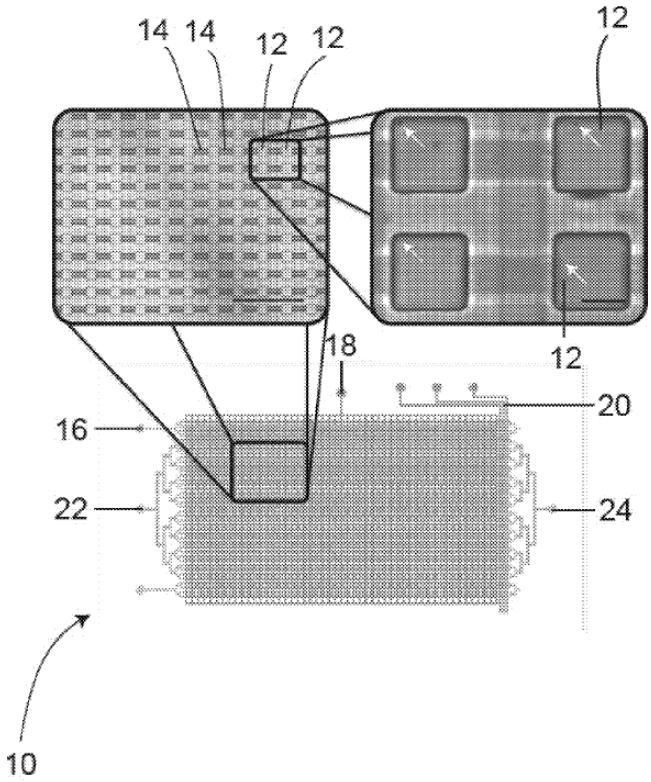
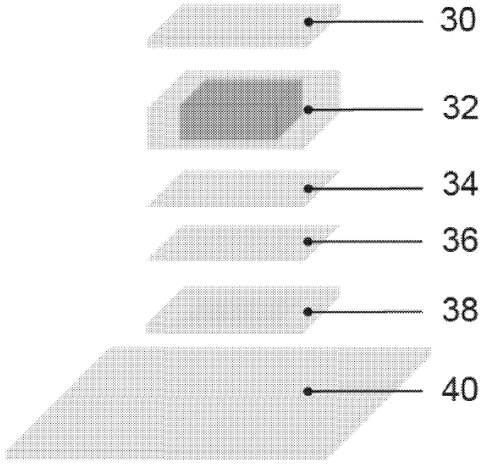


FIGURE 1

Device 10, as depicted in Figure 1 above, comprises an array of 1,600 chambers 12, each "with integrated microvalves to allow precise control and exchange of media." Ex. 1001, 23:12–13. Fluid enters chambers 12 from array inlet 22 via flow channels 14, and fluid exits through array outlet 24. *Id.* at 23:13–14, 23:18–21. To control cell loading and perfusion rates, device 10 also includes control lines 18 (connected to an isolation valve) and 20 (connected to a peristaltic pump). *Id.* at 23:16–18. Hydration lines 16 are located on each side of the array to minimize edge effects. *Id.* at 23:14–15. In the high-resolution view (shown in top right of Figure 1), arrows point to the location of single cells in each chamber 12. *Id.* at 23:21–22.

Figure 2, reproduced below, is an exploded view of device 10 showing the layers of the device as they are assembled during fabrication. Ex. 1001, 6:22–24, 23:44–46.



As shown above in Figure 2, device 10 includes the following layers (from bottom to top): glass slide 40; chamber layer 38, which includes chambers 12 (not shown); control layer 36, which includes control structures; a 150 μm-thick PDMS[1] membrane 34; iso-osmotic bath layer 32 consisting of a large chamber "filled with medium to prevent evaporation and maintain constant osmolarity inside the chambers [12]"; and "gas-permeable PDMS cover layer 30 to keep the [iso-osmotic] bath sterile." Ex. 1001, 23:46–63.

Figure 4 of the '408 patent, reproduced below, is a cross-sectional view of chamber 12 and channel 14 of microfluidic device 10 while chamber 12 is being perfused with a fluid. Ex. 1001, 6:28–32.

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[1] According to the '408 patent, PDMS is an abbreviation for polydimethylsiloxane, "a transparent and biocompatible silicone elastomer . . . widely used for cell-culture applications," which "provide[s] high gas permeability for the efficient exchange of oxygen and carbon dioxide." Ex. 1001, 2:38–42.

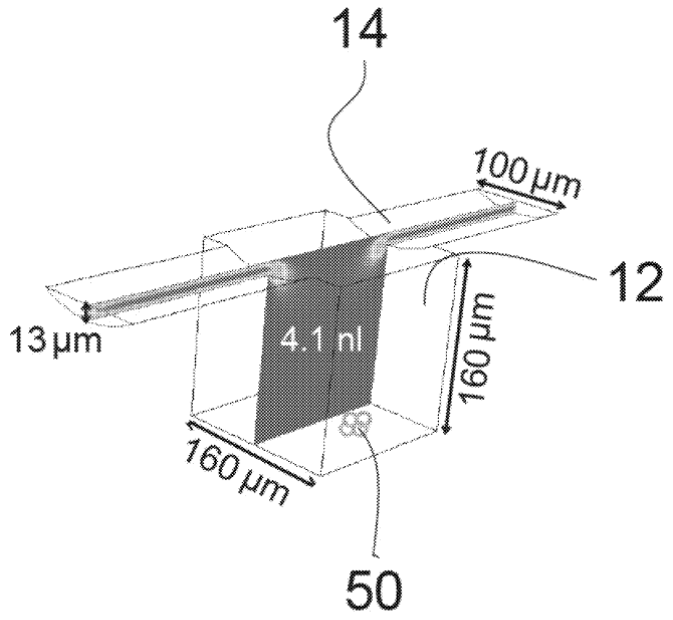

FIGURE 4

Figure 4, above, depicts culture chamber 12 (a cube of 160 µm on each side, forming a volume of about 4.1 nl) and flow channel 14 (100 µm wide and up to 13 µm deep). Ex. 1001, 24:39–40, 24:64–65, 26:20–22. Chamber 12 includes trapped cells 50. *Id.* at 24:36. The '408 patent discusses a numerical simulation that predicts a "sudden expansion when the fluid moves from the flow channel [14] to the chamber [12 that] creates a velocity drop," and "minimal flow rates at the bottom 5/6 of the chamber." *Id.* at 26:12–15, 26:23–24. "[T]he gravitational forces on the cells [are] greater than hydrodynamic forces and cells remain in the cell retaining region while the perfusion fluid exits the chamber through the flow channel outlet." *Id.* at 26:24–28.

After culturing cells 50 in chambers 12, the user can recover them "by simply inverting the device, causing the cells to settle into the higher-flow rate regions of the chambers (as shown in FIG. 4) and then recovering the pooled population by flushing back through the input port." Ex. 1001,

26:50–53. Alternatively, "when selective recovery of the contents of specific individual wells is desired, the layer of PDMS [30 as shown in Fig. 2] covering the osmotic bath [32] can first be removed and a sterile micropipette then used to pierce the membrane [34] over any selected chamber followed by aspiration of its contents." *Id.* at 26:57–62.

C. CHALLENGED CLAIMS AND GROUNDS

Claim 1, representative of the challenged claims, reads as follows:

1. A method of culturing a cell, the method comprising:

[A] retaining the cell at a retaining position within an individual chamber of a microfabricated device;

[B] perfusing the cell with a perfusion fluid by flowing the perfusion fluid into the individual chamber through an inlet and out of the chamber through an outlet,

[C] wherein the outlet is positioned such that gravitational forces acting on the cell to keep it at or near the retaining position exceed hydrodynamic forces acting on the cell to move it toward the outlet;

[D] culturing the cell within the chamber and monitoring a response in the chamber; and

[E] selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step.

Ex. 1001, 31:62–32:9 (Petitioner's reference letters added).

Petitioner argues five grounds for *inter partes* review, as summarized in the following table:

Ground	Claim(s) Challenged	35 U.S.C. §[2]	Reference(s)/Basis
1	1, 6, 11, 19, 24, 26, 27, 30	102(a), (e)	Dimov[3]
2	1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov
3	1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov, Kovac[4]
4	1, 11, 16, 24, 26, 27, 30	103(a)	Park[5]
5	1, 11, 16, 24, 26, 27, 30	103(a)	Park, Kovac

Pet. 8.

D. DECLARATORY TESTIMONY

Petitioner relies on two declarations of Dr. Carl Meinhart. Exs. 1002, 1039. Petitioner also relies on the declaratory testimony of Dr. Ingrid Hsieh-Yee to establish the date of public availability for certain references. Ex. 1020.

Patent Owner relies on a declaration of Dr. Bruce K. Gale. Ex. 2012.

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[2] 35 U.S.C. §§ 102, 103 (2006), *amended by* Leahy–Smith America Invents Act, Pub. L. No. 112-29 §§ 102, 103, sec. (n)(1), 125 Stat. 284, 287, 293 (2011) (effective Mar. 16, 2013). These versions of §§ 102 and 103 apply because the effective priority date of the '408 patent (no later than July 7, 2011, *see* Ex. 1001, code (22)) is before the effective date of the AIA amendments.

[3] Dimov et al., US 8,906,669 B2 (issued Dec. 9, 2014 from application filed Oct. 9, 2009) (Ex. 1003). Petitioner also includes, within this ground, published international and US versions of the application which, according to Petitioner, contain the same text as all citations to Dimov in the Petition. Pet. 6 & n.1 (citing Exs. 1004, 1005). We refer to these publications, collectively, as "Dimov."

[4] J.R. Kovac & J. Voldman, *Intuitive, Image-Based Cell Sorting Using Optofluidic Cell Sorting*, 79 Anal. Chem. 9321 (2007) ("Kovac," Ex. 1007).

[5] Joong Yull Park et al., *Single Cell Trapping in Larger Microwells Capable of Supporting Cell Spreading and Proliferation*, 8 Microfluidics & Nanofluidics 263 (2010) ("Park," Ex. 1006).

III. GROUNDS OF THE PETITION

For the reasons below, we determine that Petitioner has not shown, by a preponderance of the evidence, that any claims of the '408 patent are unpatentable under the grounds of the Petition. Before analyzing these grounds in detail, we address two matters that will underlie our analysis: the level of ordinary skill in the art and the construction of the claim terms.

A. LEVEL OF ORDINARY SKILL IN THE ART

The level of ordinary skill in the pertinent art at the time of the invention is a factor in how we construe patent claims. *See Phillips v. AWH Corp.*, 415 F.3d 1303, 1312–1313 (Fed. Cir. 2005) (en banc). It is also one of the factors we consider when determining whether a patent claim would have been obvious over the prior art. *See Graham v. John Deere Co.*, 383 U.S. 1, 17–18 (1966).

To assess the level of ordinary skill, we construct a hypothetical "person of ordinary skill in the art," from whose vantage point we assess obviousness and claim interpretation. *See In re Rouffet*, 149 F.3d 1350, 1357 (Fed. Cir. 1998). This legal construct "presumes that all prior art references in the field of the invention are available to this hypothetical skilled artisan." *Id.* (citing *In re Carlson*, 983 F.2d 1032, 1038 (Fed. Cir. 1993)).

Relying on Dr. Meinhart's testimony, Petitioner argues that a person of ordinary skill in the art "would have had a bachelor's degree in mechanical engineering, chemical engineering, biomedical engineering, molecular biology, (bio)chemistry, or an equivalent degree relevant to microfluidic biological cell analysis, and three to five years of experience with the construction of and application of microfluidic devices to cell

culture and analysis." Pet. 8–9 (citing Ex. 1002 ¶ 40). Patent Owner does not contest Petitioner's proposed level of ordinary skill in the art. *See* PO Resp. 3–4 (citing Ex. 2012 ¶¶ 19–20).

We find Petitioner's uncontested articulation to be reasonable in light of the subject matter involved in the '408 patent and its description of the relevant prior-art background. *See, e.g.*, Ex. 1001, 1:15–2:54 (describing the field and related art as relating to the construction and use of microfluidic devices in cell culture and analysis). Thus, we adopt it for our decision.

B. CLAIM CONSTRUCTION

In an *inter partes* review, we construe a patent claim "using the same claim construction standard that would be used to construe the claim in a civil action under 35 U.S.C. 282(b)." 37 C.F.R. § 42.100(b) (2020). This generally includes "construing the claim in accordance with the ordinary and customary meaning of such claim as understood by one of ordinary skill in the art and the prosecution history pertaining to the patent." *Id.* "[W]e look principally to the intrinsic evidence of record, examining the claim language itself, the written description, and the prosecution history, if in evidence." *DePuy Spine, Inc. v. Medtronic Sofamor Danek, Inc.*, 469 F.3d 1005, 1014 (Fed. Cir. 2006) (citing *Phillips*, 415, F.3d at 1312–1317).

The ordinary and customary meaning of a claim term "is its meaning to the ordinary artisan after reading the entire patent," and "as of the effective filing date of the patent application." *Phillips*, 415 F.3d at 1313, 1321. There are only two circumstances in which we adopt a construction that departs from the ordinary and customary meaning: "1) when a patentee sets out a definition and acts as [their] own lexicographer, or 2) when the

patentee disavows the full scope of a claim term either in the specification or during prosecution." *Thorner v. Sony Comput. Entm't Am. LLC*, 669 F.3d 1362, 1365 (Fed. Cir. 2012). Any such special meaning of a term "must be sufficiently clear in the specification that any departure from common usage would be so understood by a person of experience in the field of the invention." *Multiform Desiccants Inc. v. Medzam Ltd.*, 133 F.3d 1473, 1477 (Fed. Cir. 1998).

Petitioner contends that "no terms require express construction for purposes of resolving the challenges in this proceeding." Pet. 9; *accord* Pet. 12. Petitioner also notes that in the parallel district court proceedings, Patent Owner has proposed constructions for the terms *chamber* and *retaining position* that differ from Petitioner's proposed constructions. Pet. 9–10 (citing Ex. 1013 App'x A, 1, 3 (Patent Owner's proposed constructions); Ex. 1021, 7, 8 (Petitioner's proposed constructions); Ex. 1002 ¶ 44). In those proceedings, Patent Owner's proposed construction for *chamber* is "an enclosed space within a microfluidic device," and its proposed construction for *retaining position* is "a location in the chamber at which a cell is maintained during cell culture and media exchange." Ex. 1013, 6, 8. Although Petitioner does not contend that its arguments rely on the choice of construction for these terms, Petitioner adopts Patent Owner's constructions for this proceeding. Pet. 11. Patent Owner does not contest these proposed constructions, or propose any other explicit constructions. *See generally* PO Resp.

We agree with Petitioner that we do not need to construe the terms *chamber* and *retaining position* to decide the issues presented in this proceeding, and to the extent we need to interpret the meaning of any claim

language, we address the interpretations below in the context of the prior art. *See Nidec Motor Corp. v. Zhongshan Broad Ocean Motor Co.*, 868 F.3d 1013, 1017 (Fed. Cir. 2017) ("[W]e need only construe terms 'that are in controversy, and only to the extent necessary to resolve the controversy' . . . ." (quoting *Vivid Techs., Inc. v. Am. Sci & Eng'g, Inc.*, 200 F.3d 795, 803 (Fed. Cir. 1999))).

C. GROUNDS BASED ON DIMOV ALONE (GROUNDS 1 AND 2)

In Grounds 1 and 2, Petitioner contends that Dimov anticipates claims 1, 6, 11, 19, 24, 26, 27, and 30, or alternatively that the claims are obvious over Dimov. *See* Pet. 12–40.

1. Overview of Dimov

Dimov describes "a microfluidic device having a fluid path defined within a substrate between an input and an output," such that "particles provided within a fluid flowing within the fluid path will preferentially collect within the capture chamber." Ex. 1003, code (57).

Figure 2, reproduced below, illustrates the overall microfluidic device:

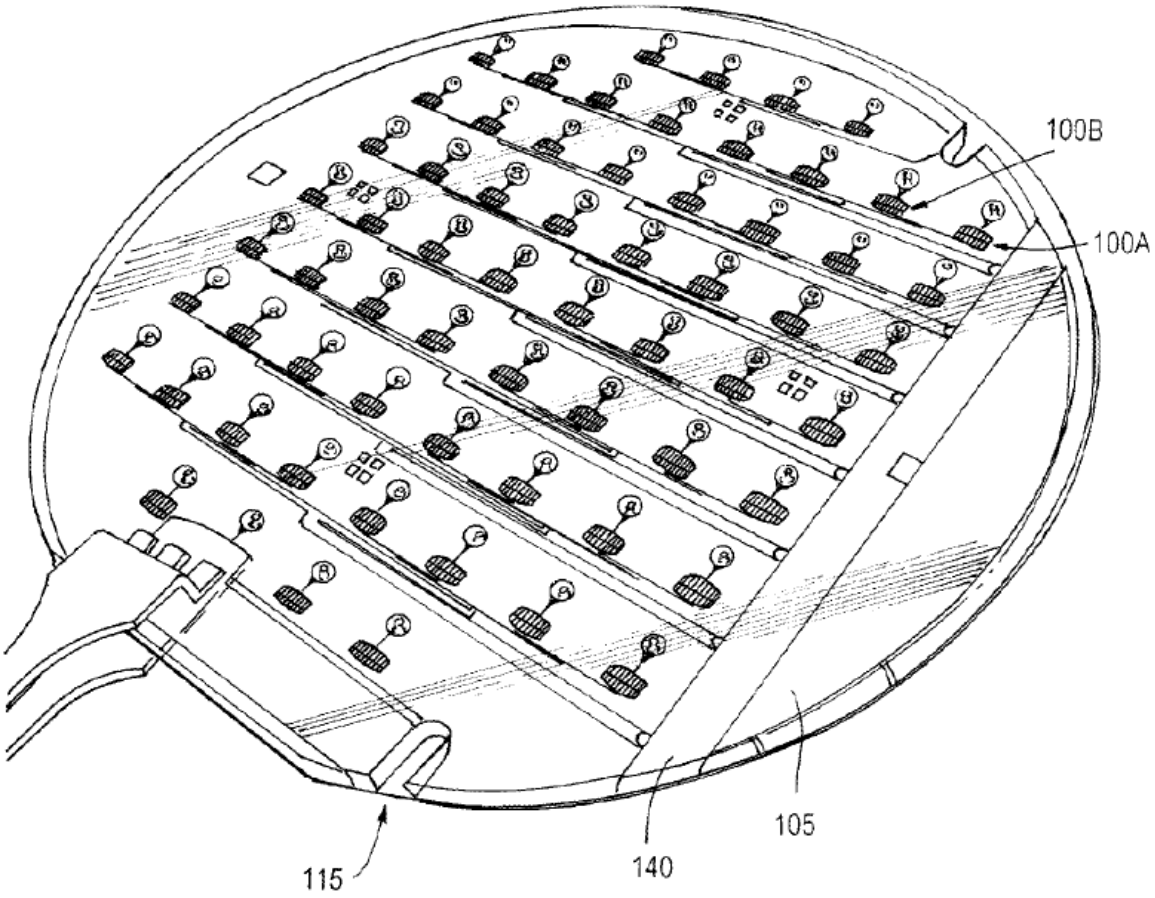

FIG. 2

Figure 2, above, depicts identical microfluidic devices 100 (100A, 100B, and many others) formed within substrate 105, and a multiplexed structure by which waste fluids are collected in common waste 140. *See* Ex. 1003, 2:29–30, 3:22–24, 3:54–57, 4:20–22. In use, substrate 105 is situated horizontally. *See id.* at 3:37–41.

Figure 1, reproduced below, shows microfluid device 100 (A or B) in more detail:

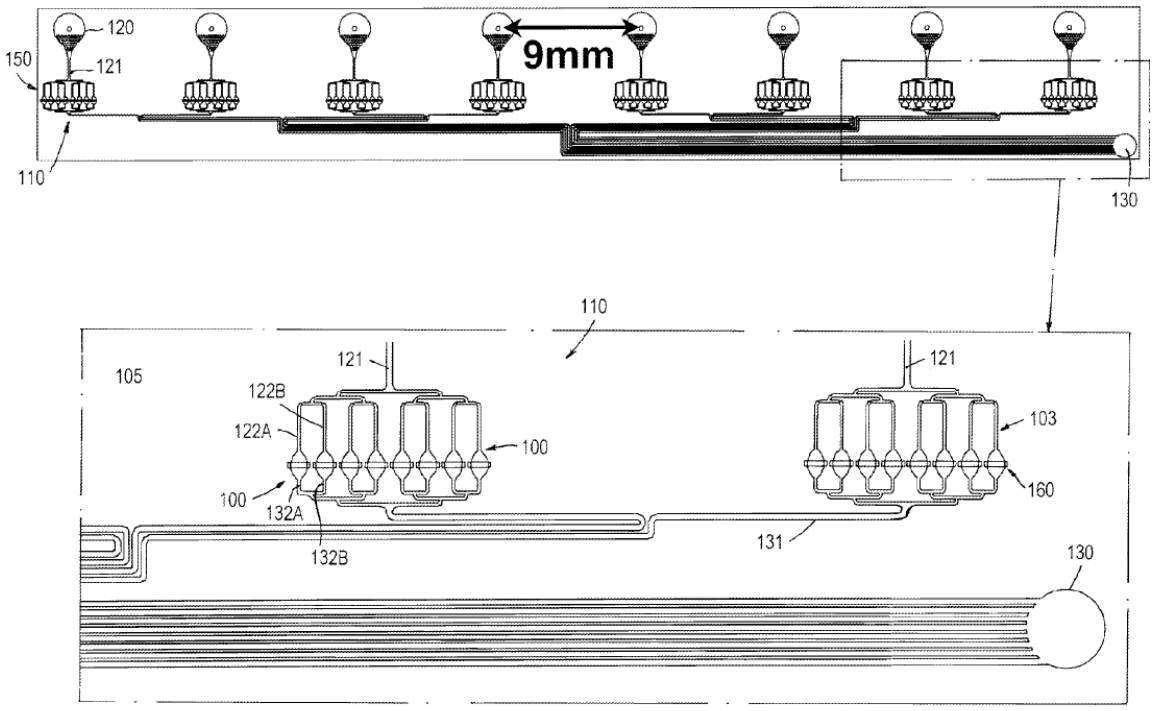

FIG. 1

As shown in Figure 1, above, each microfluidic device 100 "comprises a fluid path 103 defined within a substrate 105 between an input 120 and an output 130." Ex. 1003, 3:24–26. Capture chambers 160 are also situated within fluid paths 103 and extend perpendicularly into substrate 105 so that cells passing through fluid path 103 are captured in chambers 160 by the downward pull of gravity. *See id.* at 3:27–53. Outputs 130 for each row empty into common waste 140 (shown above in Figure 2). *Id.* at 4:20–22.

Figure 4B, reproduced below, shows an individual capture chamber 160 within the microfluidic device:

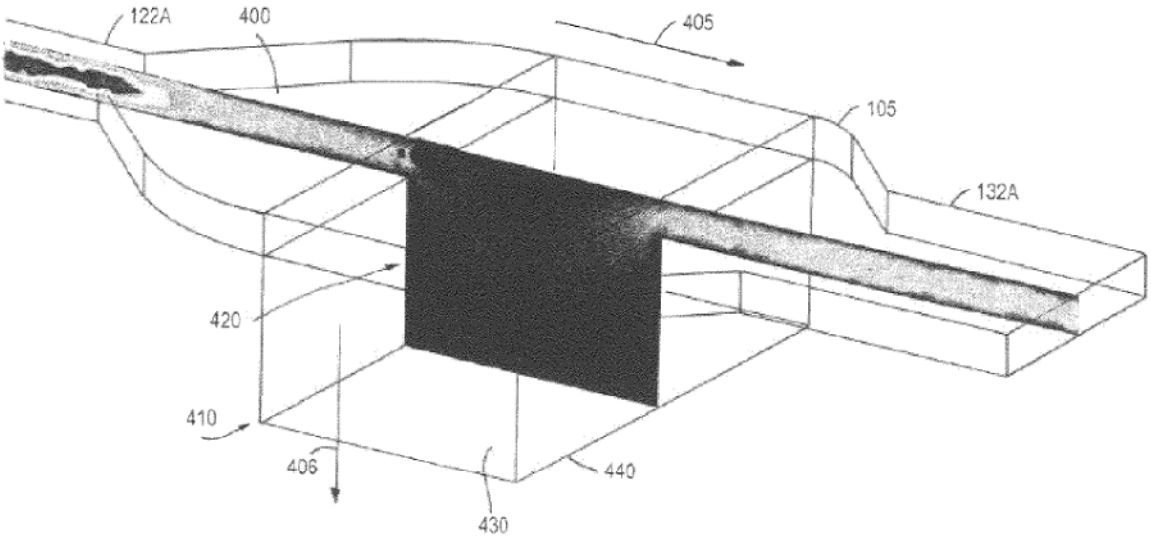

FIG. 4B

The capture chamber shown in Figure 4B, above, includes trench 410, formed within substrate 105, with mouth 420 in communication with fluid path 400, and sidewalls 430 that extend downward from mouth 420 to base 440. Ex. 1003, 5:27–32.

Dimov's trench 410 traps cells because "[a]s the fluid passes over the mouth of the trench it enters downwardly into the trench. This . . . causes a deceleration of the fluid," which "causes particles within the fluid to be displaced from the fluid. Once displaced, they settle towards the base 440." Ex. 1003, 6:37–43. Dimov teaches that "the trench is desirably dimensioned relative to the flow rate of the operating conditions such that once displaced the particles will be retained within the trench." *Id.* at 6:44–47.

Dimov teaches that "the dimensions of the capture trench are much greater than the particles which are retained therein." Ex. 1003, 11:3–4. In one embodiment, its depth is approximately 300 μm. *Id.* at 5:39–45. In another embodiment, its horizontal length is 200 μm. *See id.* at 6:57–62, Fig. 5. In yet another embodiment, its horizontal size is 100×400 μm with a

depth of 320 μm, *id.* at 9:16–17. Dimov teaches that one benefit of these relatively large dimensions is that the device "can be usefully employed in biomimetic experiments" such as three-dimensional layered structures. *Id.* at 11:5–21.

An example of such three-dimensional layered structures is shown in Figure 21, reproduced below:

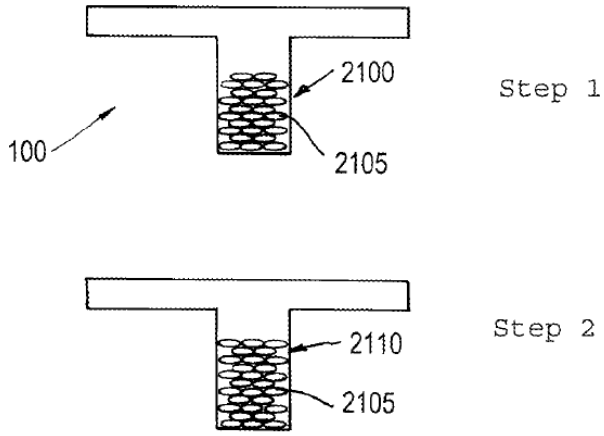

FIG. 21

Figure 21 of Dimov, above, depicts device 100 that "can be used to generate 3D cell structures 2100 of individual cancer cells 2105 so as to recreate cellular conditions similar to in-vivo tumours or other structures." Ex. 1003, 11:7–10. According to Dimov, layered cells 2105 are typically "retained in the order that they were introduced into the chamber," which "allows for subsequent experiments to be conducted within pseudo in vivo conditions." *Id.* at 11:19–21.

Dimov states that "the arrangements described herein preferentially retain the particles within the trench." Ex. 1003, 11:21–23. Alternatively, Dimov also teaches that

> [i]t is possible to modify the arrangement so as to provide for subsequent movement of the particles—either within the trench so as to provide for mixing or the like, *or to effect removal of the particles out of the trench*. Such arrangements will typically require a capacity to manipulate the particles and this can be conducted either before or subsequent to capture of the particles within the trench. Examples of techniques that could be employed include:
>
> Acoustic
> Magnetic
> Inertial
> Electric
> Dielectrophoretic
> Thermo-hydrodynamic
> *Laser tweezers*
> Hydrodynamically induced agitation
> Specific or unspecific attachment to surface
>
> It will be understood that the use of such techniques may require an external source of agitation or manipulation of the particles.

Id. at 11:23–43 (emphasis added). Thus, Dimov lists a number of techniques, including "[l]aser tweezers," for mixing or removing particles in a trench. *See id.*

2. Uncontested Limitations 1A–D

Petitioner contends that Dimov discloses the preamble and limitations 1A–D, and points to passages within Dimov describing retaining a cell in a chamber, perfusing the cell with fluid while the cell is retained in the chamber, and culturing the cell within the chamber while monitoring a response. Pet. 13–26 (citing Ex. 1003, 1:40–47, 1:52–53, 1:57–60, 2:25–27, 2:33–36, 3:37–41, 3:47–53, 5:10–24, 5:27–35, 6:37–47, 7:4–20, 7:24–28, 8:16–18, 8:40–42, 9:32–48, 10:10–11, 10:55–58, 12:13–19, 12:66–13:3,

13:11–18, 13:23–42, 13:59–67, 14:1–6, 14:8–10, 14:13–17, 14:26–56, 15:1–13, 15:20–26, 16:4–7, Figs. 4B, 6, 7, 24; Ex. 1002 ¶¶ 54–84).

Patent Owner does not dispute Petitioner's allegation that Dimov discloses limitations 1A–D. *See generally* PO Resp. We need not address Petitioner's allegations regarding the preamble or limitations 1A–D because we determine, below, that Dimov does not disclose or teach limitation 1E.

3. Asserted Anticipation by Dimov

Limitation 1E recites "selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step." Ex. 1001, 32:7–9. In arguing that Dimov anticipates claim 1, Petitioner contends that Dimov discloses limitation 1E by listing various techniques, such as "[l]aser tweezers," that can be used "within the trench to provide for mixing or the like, *or to effect removal of the particles out of the trench*." Pet. 27 (quoting Ex. 1003, 11:21–26) (citing Ex. 1002 ¶ 85).

Petitioner argues that because "Dimov refers to removal of the particles out of an individual trench ('the trench'), . . . Dimov discloses selectively recovering the individual cell, or a clonal population thereof, from the individual chamber." Pet. 28 (citing Ex. 1002 ¶ 88). According to Petitioner, a person of ordinary skill in the art "would have understood that at least some of the [listed] techniques, such as laser tweezers, are techniques that, by their very natures and because of their technical limitations, would necessarily be directed to individual trenches." Pet. 28 (citing Ex. 1002 ¶ 89).

Petitioner also argues that Dimov motivates "subsequent analysis or experimentation" within the chamber after loading the cells into the device,

and a person of ordinary skill in the art would have had the same motivation to remove the cells from the device for later analysis or experimentation. Pet. 29 (quoting Ex. 1003, 12:18) (citing Ex. 1003, 11:29–30, 12:13–18; Ex. 1002 ¶ 90). According to Petitioner, a person of ordinary skill "would have at once envisaged basing the removal of the cell on the response in a monitoring step." Pet. 29 (citing *Kennametal, Inc. v. Ingersoll Cutting Tool, Co.*, 780 F.3d 1376, 1381 (Fed. Cir. 2015); Ex. 1002 ¶ 91); *see also* Pet. 29–30 (citing Ex. 1002 ¶¶ 92–92) (giving examples of reasons why a person of ordinary skill would have envisioned removing cells for further analysis).

In its Response, Patent Owner contends that Dimov fails to disclose either "selectively recovering cells" or a "mechanism by which a cell could be recovered." PO Resp. 5 (citing Ex. 2012 ¶¶ 24–38, 58–68). Patent Owner argues that all the specific examples in Dimov are directed to the embodiment in which cells are retained in the trenches and analyzed by exposing them to different fluids and conditions while they are still in the trenches. *See id.* at 5–6 (citing Ex. 1003, 7:30–39, 8:4–9, 10:11–12, 10:55–58, 11:1–21, 12:13–18, 13:43–46; Ex. 2010, 161:11–24; Ex. 2012 ¶¶ 23–37, 58–69). Indeed, according to Patent Owner, Dimov contemplates that cell responses will be analyzed by exposing the cells "'to a suitable lysis agent' in the trenches so that they burst and 'their contents may be released' into the liquid media." *Id.* at 7 (citing Ex. 1003, 7:37–41, 8:6–9; Ex. 2010, 173:21–174:5; Ex. 2012 ¶ 63).

Patent Owner also argues that Dimov fails to disclose, within its microfluidic device, any structure that would allow for the selective recovery of a cell or clonal cell population. PO Resp. 6–7. According to Patent Owner, "there is no direct access to the trenches, and all fluid leaving every

trench converges into a single common waste, from which cells cannot be selectively recovered." *Id.* at 7 (citing Ex. 1003, 4:5–8, 4:18–22; Ex. 2012 ¶¶ 58–69); *see also id.* at 29–32. Relying on the testimony of Dr. Gale, Patent Owner argues that the lines leading out of each capture chamber are "very long and convoluted, which introduces additional complications such as loss of cells to interior walls, an inability to follow any specific cells of interest, significant dilution, shear stresses, and congestion, none of which support cell recovery." *Id.* at 7 (citing Ex. 2012 ¶¶ 24, 30, 63). Further, to the extent that lysis agents are used in any of the experiments on the chip, Patent Owner contends that such agents would destroy any intact cells on their path to the common waste. *Id.* at 7–8 (citing Ex. 1003, 4:1–22, Fig. 1; Ex. 2010, 173:1–20; Ex. 2012 ¶¶ 32, 60–62).

Finally, Patent Owner argues that, while Dimov briefly discusses "removal" of particles from the trenches and a "generic list" of nine techniques for mixing or removal, the focus of Dimov "is on what is retained in the trench, not what is discarded or removed." PO Resp. 8 (citing Ex. 2012 ¶¶ 59–69); *see also* Ex. 2012 ¶ 64 (Dr. Gale opining that Dimov's mention of removal techniques relates to "removing waste or other unwanted particles out of the capture chamber/trench"[6]). According to Patent Owner, Dimov provides no disclosure, and no examples, about "(i) how specifically to employ the list of potential techniques, (ii) how to agitate or

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[6] Petitioner appears to interpret Dr. Gale's testimony in paragraph 64 of his declaration as opining that the "particles" that Dimov mentions are not cells. *See* Pet. Reply 3–4. This is not how we interpret his testimony, and in any event, Dr. Gale agreed on cross-examination that in Dimov, the term *particles* may include cells. *See* Ex. 1038, 46:4–47:10.

manipulate the particles, [or] (iii) how to remove, much less selectively recover *individual* cells without damaging or otherwise altering the cells to be removed." *Id.* at 8–9. Thus, Patent Owner disagrees with Petitioner that a person of ordinary skill in the art "would have at once envisaged" performing a selective cell recovery in response to monitoring a cell response in one of the chambers. *Id.* at 9–10 (quoting Pet. 29).

In its Reply, Petitioner contends that Patent Owner does not dispute that Dimov discloses at least some embodiments that could be used for removing cells from Dimov's individual trenches, or "that removing particles from one of multiple trenches constitutes selectively recovering the particles." Pet. Reply 2–3 (citing Ex. 1001, 26:57–62; Ex. 1003, 11:21–26; Ex. 2012 ¶¶ 37, 66; Ex. 1038, 123:17–125:10; Ex. 1039 ¶¶ 5–9). And according to Petitioner, Dimov's example of using the trenches for biomimetic experiments is not the only disclosed embodiment, and Dimov frames the possibility of cell removal as an alternative embodiment. *Id.* at 3 (citing Ex. 2010, 205:19–206:11; Ex. 1039 ¶¶ 13–20).

Finally, Petitioner argues that Dimov's disclosure is not inconsistent with selectively recovering cells because (a) Dimov's disclosure about retaining and analyzing cells in the trenches does not preclude subsequently removing the cells (Pet. Reply 4 (citing Ex. 1002 ¶ 92; Ex. 1039 ¶¶ 21–30)); (b) Dimov does not teach that every single experiment in its devices will include the use of a lysis agent (*id.* (citing Ex. 1038, 56:2–5, 55:15–20; Ex. 1039 ¶¶ 49–51)); (c) limitation 1E does not impose any requirements on what happens to a cell after it is selectively recovered (*id.* (citing Ex. 1039 ¶¶ 21–30, 52–55)); (d) none of Dimov's structural features, such as the common waste stream, "would prevent selective recovery" (*id.* at 5 (citing

Ex. 1039 ¶¶ 31–48[7]); and (e) "Patent Owner fails to show that a [person of ordinary skill in the art] would have lacked the knowledge or skill to implement Dimov's removal techniques—including laser tweezers, which was well known in the art" (*id.* (citing Ex. 1039 ¶¶ 11–12)).

To establish anticipation, a petitioner must find each and every element in a claim, arranged as recited in the claim, in a single prior art reference. *See Net MoneyIN, Inc. v. VeriSign, Inc.*, 545 F.3d 1359, 1369 (Fed. Cir. 2008). The limitations may be present in the reference "either explicitly or inherently." *In re Schreiber*, 128 F.3d 1473, 1477 (Fed. Cir. 1997). Further, an anticipating prior art reference must be enabling and must describe the "claimed invention sufficiently to have placed it in possession of a person of ordinary skill in the field of the invention." *See Helifix Ltd. v. Blok-Lok, Ltd.*, 208 F.3d 1339, 1346 (Fed. Cir. 2000). We do, however, consider the reference "together with the knowledge of one of ordinary skill in the pertinent art." *In re Paulsen*, 30 F.3d 1475, 1480 (Fed. Cir. 1994) (citing *In re Samour*, 571 F.2d 559, 562 (CCPA 1978)).

Based on these principles, we agree with Patent Owner that Petitioner has not identified within the four corners of Dimov a sufficient disclosure of

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[7] Petitioner improperly cites these eighteen paragraphs (more than ten pages) from Dr. Meinhart's reply declaration in support of this conclusory technical assertion, without any additional commentary. Our rules prohibit this type of incorporation of testimony by reference, and we do not consider the cited paragraphs in our analysis. 37 CFR 42.6(a)(3) ("Arguments must not be incorporated by reference from one document into another document."); *Cisco Sys., Inc. v. C-Cation Techs., LLC*, IPR2014-00454, Paper 12 at 9 (PTAB Aug. 29, 2014) (informative) (declining to consider "conclusory statements [in an expert declaration] for which the Petition does not otherwise provide an argument or explanation").

selectively recovering a cell based on the response in the monitoring step. Although Dimov discloses non-selective recovery and briefly lists a few general techniques that might be used for either mixing or recovery, Dimov does not specifically indicate which of the techniques would be useful for recovery rather than just mixing, and Dimov does not explicitly disclose that any cell recovery would be selective. Nor has Petitioner shown that Dimov inherently discloses selectively recovering any particular cell that has been cultured and monitored, because, as Dr. Gale persuasively testifies, Dimov's removal techniques could, as far as a person of ordinary skill in the art would have known based on Dimov's disclosure, be merely for "removing . . . unwanted particles out of the capture chamber/trench." Ex. 2012 ¶ 64; *see also id.* ¶ 63 ("Dimov . . . may suggest removing cellular materials (rather than intact cells) after capture . . . ."). In other words, given Dimov's general focus on performing experiments *within* the trenches and the lack of any specific disclosure about how or why to remove cells, Petitioner has not met its burden to show that Dimov's potential cell-removal techniques would have *necessarily* been based on a response of a specific cell being cultured and monitored in limitation 1D.

We also agree with Patent Owner that Dimov does not disclose any specifics about how to carry out a selective cell removal based on one of Dimov's listed techniques, such as laser tweezers. *See* PO Resp. 8–9. Nor does Petitioner meet its burden of showing that such a technique would have been within the ordinary skill in the art at the time of the claimed invention. "In order to anticipate, a prior art disclosure must also be enabling, such that one of ordinary skill in the art could practice the invention without undue experimentation." *Novo Nordisk Pharms., Inc. v. Bio-Tech. Gen. Corp.*, 424

F.3d 1347, 1355 (Fed. Cir. 2005) (citing *SmithKline Beecham Corp. v. Apotex Corp.*, 403 F.3d 1331, 1342 (Fed. Cir. 2005)). Given the labyrinthine outlet channels leading from Dimov's chambers to the common waste stream and Dimov's lack of any disclosure of a way to identify and extract cells in the outlet streams, we credit Dr. Gale's testimony that there would have been substantial obstacles to recovering any specific cells that had been liberated from a trench in Dimov's device. *See* Ex. 2012 ¶ 63. Petitioner has not shown that a person of ordinary skill in the art would have overcome those obstacles without undue experimentation.

Thus, the evidence of record does not support Petitioner's argument that Dimov discloses limitation 1E or, consequently, that claim 1 is unpatentable under 25 U.S.C. § 102 as anticipated by Dimov.

4. Obviousness over Dimov

Alternatively, Petitioner argues in Ground 2 that claim 1 would have been obvious over Dimov, in view of the background knowledge of a person of ordinary skill in the art. Pet. 34–35 (citing Ex. 1002 ¶ 106).

A claim is unpatentable under § 103(a) for obviousness if the differences between the claimed subject matter and the prior art are "such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains." *KSR Int'l Co. v. Teleflex Inc.*, 550 U.S. 398, 406 (2007). When a ground in a petition is based on a combination of references, we consider "whether there was an apparent reason to combine the known elements in the fashion claimed by the patent at issue." *Id.* at 418 (citing *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006)).

We base our obviousness inquiry on factual considerations including (1) the scope and content of the prior art, (2) any differences between the claimed subject matter and the prior art, (3) the level of skill in the art, and (4) any objective indicia of obviousness or non-obviousness that may be in evidence.[8] *See Graham*, 383 U.S. at 17–18.

The main issue in dispute for this ground is whether there was a sufficient motivation and sufficient background knowledge in the art, at the time of the claimed invention, that a person of ordinary skill in the art would have modified Dimov's device to introduce selective recovery as recited in limitation 1E. For the reasons below, we determine that Petitioner has not met its burden of persuasion on this issue by a preponderance of the evidence.

According to Petitioner, Dimov discloses both monitoring a response in the chamber and removing cells from the chamber, and persons of ordinary skill in the art would have been motivated to selectively remove cells based on the monitored response, as recited in limitation 1E, so that they could further investigate cells of interest cultured in the chamber (such as to sequence their DNA or to expand the cells by culturing). Pet. 35–36 (citing Ex. 1002 ¶¶ 107–110).

As with its anticipation ground, Petitioner contends that an ordinarily skilled artisan would have removed cells using used one of the techniques listed in Dimov, such as laser tweezers. Pet. 36 (citing Ex. 1003, 11:30–40).

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[8] Because neither party argues that there are objective indicia of obviousness or non-obviousness, this does not factor into our decision. *See generally* Pet.; PO Resp.; Pet. Reply; PO Sur-Reply.

In an alternative proposed modification, Petitioner argues that a person of ordinary skill in the art would have inserted a micropipette into a trench to aspirate one or more of the cells of interest for further analysis. Pet. 36 (citing Ex. 1003, 11:30–40; Ex. 1002 ¶ 111). Below, we address each of these alternative theories in turn.

(a) Modification of Dimov by Using Listed Removal Techniques

Petitioner's first obviousness theory is that a person of ordinary skill in the art would have had reason to use one of the listed cell-removal techniques, such as laser tweezers, for selective cell recovery according to limitation 1E. *See* Pet. 36. As we note above, Petitioner asserts that the motivation for this modification would have been to conduct further investigations on the cells, which may, for example, involve sequencing or culturing. *See* Pet. 35–36 (citing Ex. 1002 ¶¶ 107–110).

In response, Patent Owner contends that, if a person of ordinary skill in the art wanted to conduct further investigations on cells outside of a fluidic device, the evidence does not show that they would have started with Dimov's "lab on a chip," which "was not designed for selective cell recovery, but rather to capture cells in its chambers and retain them there for further analysis, and there is no mechanism by which a cell could be recovered." PO Resp. 11–12 (citing Ex. 1003, 7:30–37; Ex. 2010, 159:2–160:22 (Dr. Meinhart acknowledging that Dimov is an example of lab-on-a-chip technology); Ex. 2012 ¶¶ 23–37, 70–81); *see also id.* at 18 (citing Ex. 2012 ¶¶ 87–90). Moreover, Patent Owner contends that any motivation to conduct subsequent experiments on cells of interest is already met by Dimov's teaching that subsequent experiments take place in the same trench,

without removing them. *Id.* at 21–22 (citing Ex. 1003, 8:8–10, 14:1–15:26; Ex. 2010, 186:22–187:2; Ex. 2012 ¶¶ 24–37, 58–69, 79–80, 87–118).

To allow for selective recovery, Patent Owner contends that a person of ordinary skill in the art would have had to essentially redesign Dimov's device to allow for external access to the trenches. PO Resp. 12; *see also id.* at 13 (arguing that the Examiner accepted a similar argument about Dimov during prosecution). According to Patent Owner, persons of ordinary skill in the art would not have made such a modification unless we attribute them with impermissible hindsight. *Id.* at 14 (citing *Cheese Sys., Inc. v. Tetra Pak Cheese & Powder Sys., Inc.*, 725 F.3d 1341, 1352 (Fed. Cir. 2013)); *see also id.* at 17 (citing Ex. 2012 ¶¶ 88–90; *InSite Vision, Inc. v. Sandoz, Inc.*, 783 F.3d 853, 859–60 (Fed. Cir. 2015) ("Defining the problem in terms of its solution reveals improper hindsight in the selection of the prior art relevant to obviousness.")).

Patent Owner also argues that, while Dimov mentions removal of particles from the trenches, Dimov's main focus is conducting experiments, and in particular, biomimetic experiments, fully within the trenches, and Dimov does not give any examples of selective cell recovery for further investigation. PO Resp. 18–19 (citing Ex. 1003, 7:30–37, 11:1–15, 11:21–43, Fig. 21; Ex. 2010, 159:2–160:22, 184:3–9 (Dr. Meinhart agreeing that Dimov does not provide specific examples of successful selective recovery of a cell), 206:12–18 (Dr. Meinhart agreeing that Dimov's discussion of cell

removal occurs within the discussion of biomimetic experiments such as shown in Dimov's Figure 21[9]); Ex. 2012 ¶¶ 23–37, 87–90, 109–123).

Thus, according to Patent Owner, Dimov does not actually teach that its listed cell mixing or removal techniques would have been for further investigation of cells exhibiting a particular response. PO Resp. 19 (citing Ex. 1003, 11:1–29; Ex. 2012 ¶¶ 112–118). Rather, given the context, Patent Owner contends that the mixing or removal techniques would have been used to adjust the cell layers during biomimetic experiments; "[f]or example, cells placed in layers for the biomimetic experiments may have needed to be rearranged because they were out of place, or because the researchers wanted a new arrangement for additional experiments." *Id.* (citing Ex. 2012 ¶¶ 112–118).

Patent Owner also argues that, because Dimov's device channels the outlet of hundreds of additional capture chambers toward a common waste line, and describes no other output mechanism, a person of ordinary skill in the art would not have considered Dimov's device to be a viable starting-point for a method of selective cell recovery, particularly if any of the concurrently running experiments involved the use of lysis agents. PO Resp. 19–21 & nn.4–5 (citing Ex. 1003, 1:30–36, 4:1–22, 4:50–61, 7:37–41, 8:6–9; Ex. 2010, 163:8–23, 173:1–174:5, 178:9–179:7, 179:13–23; Ex. 2012 ¶¶ 63, 92–99); *see also supra* Section III.C.3.

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[9] Petitioner points out that in the same cross-examination testimony, Dr. Meinhart "testified that the discussion of cell removal is *not limited to* biomimetic experiments." Pet. Reply 3 (emphasis added) (citing Ex. 2010, 205:19–206:11; Ex. 1039 ¶¶ 13–20).

In its Reply, Petitioner disagrees that Dimov's mention of cell removal is limited to biomimetic experiments, even though both discussions occur in the same paragraph. Pet. Reply 3. According to Petitioner, Dimov's discussion of mixing or removal techniques "begins with general reference to 'the arrangements described herein,' i.e., in the specification, not just the immediately preceding discussion." *Id.* (quoting Ex. 1003, 11:21–22).

Petitioner also disagrees that a person of ordinary skill in the art would not have started with Dimov to obtain a microfluidic device that can allow for selective recovery as recited in limitation 1E. *See* Pet. Reply 6–7. According to Petitioner, it is undisputed that Dimov "was designed to conduct monitoring and analysis within the device, and reported doing so successfully." *Id.* at 7 (quoting PO Resp. 12) (citing Ex. 1039 ¶¶ 57–59).

As we discuss above in the context of Petitioner's anticipation ground, we disagree with Petitioner that Dimov itself discloses limitation 1E. *See supra* Section III.C.3. We also find that Dimov's limited reference to removal of particles from a trench would have been insufficient to teach a person of ordinary skill in the art to effectively recover a cell if their motivation is, as Petitioner alleges, to conduct further investigations on the cells by, for example, sequencing or culturing. *See* Pet. 35–36.

First, we agree with Patent Owner that Dimov's device is not, without substantial modification that Dimov itself does not teach, designed for selective cell recovery. As Patent Owner persuasively argues, Dimov's device is a "lab on a chip" designed in general to retain cells while performing multiple experiments, so Dimov's design choices reflect that general purpose. *See* PO Resp. 11–12, 18, 21–22; *see also* Ex. 1003, 10:55–67 (teaching the benefits of "lab on a chip" technology which allows a user

to conduct sequential investigations within a single structure). This is, in particular, evidenced by the multiplexed outlet streams, which channel all outlets to a common waste stream 140 without any apparent regard for viable cell recovery. *See* Ex. 1003, Figs. 1–3.

In light of this design, Petitioner has not persuasively shown that a person of ordinary skill in the art would have inferred, from Dimov's brief reference to "removal of the particles out of the trench," a teaching to conduct further analyses outside of the device (such as culturing or sequencing) on cells determined to be of interest based on a prior analysis. Given that Dimov's disclosure focuses on conducting sequential experiments on the same chip, we credit Dr. Gale's testimony that a person of ordinary skill in the art may *at least* as plausibly have interpreted this passage to suggest "removing waste or other unwanted particles out of the capture chamber/trench." Ex. 2012 ¶¶ 64–65; *see also* Ex. 1038, 46:18–47:4 (testifying that Dimov also discloses the use of beads within the trenches, which are also "particles"). Petitioner has not pointed to anything in Dimov specifically suggesting that the "particles" to be removed are cells of interest destined for later analysis.

Even if Dimov does suggest removing cells to conduct further analyses outside the device, the reference merely lists a set of techniques and provides no guidance on how to carry out such removal in a way that would still allow for further meaningful analysis of cells. *See* Ex. 1003, 11:21–43. Although Dimov mentions "laser tweezers" as part of its list, Dimov does not address the specifics of liberating a particular cell or its clonal population from the device using a technique such as laser tweezers. Nor is Dimov's mention of laser tweezers sufficient to motivate a person of

ordinary skill in the art to remove cells from a trench for further analysis outside the device. Dimov also does not address the difficulties a person of ordinary skill in the art would face in finding and recovering a cell that has been ejected into Dimov's common waste stream.

Based on these considerations, we conclude that Petitioner has not persuasively shown that Dimov's brief mention of cell removal techniques provides sufficient motivation or teachings for a person of ordinary skill in the art to selectively recover cells of interest for further analysis outside the device.

(b) Modification of Dimov to Allow for Direct Access via Micropipette

Alternatively, Petitioner argues that, based on background knowledge in the art, a person of ordinary skill in the art would have modified Dimov's device "in a way that would permit the top layer of the device to be peeled back so a trench could be accessed directly." Pet. 36–37 (citing Ex. 1002 ¶ 112). According to Petitioner, Dimov already discloses that its device is constructed in two PDMS layers, and the modification would involve making these two layers "at least partially unbonded, permitting [the top layer] to be peeled back so as to allow direct access to the trench." Pet. 37 (citing Ex. 1003, 3:10–12, 5:37–39, 15:27–46, Fig. 25; Ex. 1002 ¶¶ 113–114). Petitioner also contends that Dimov does not actually teach bonding the two layers together. Pet. 38 (citing Ex. 1003, 15:44–45; Ex. 1002 ¶¶ 114–115).

For this peelable-layer embodiment, Petitioner also relies on Chao Han, et al., *Integration of Single Oocyte Trapping, In Vitro Fertilization and Embryo Culture in a Microwell-Structured Microfluidic Device*, 10 Lab on a

Chip 2848 (2010) (Ex. 1009, "Han"). Pet. 38–40. According to Petitioner, Han discloses a device with two PDMS layers, in which the upper layer can be peeled back to aspirate oocyte cells using a micropipette. *Id.* Petitioner contends that Han bears a "Received" date of May 5, 2010, which precedes the earliest filing date (July 7, 2010) to which the '408 patent claims priority. Pet. 38–39 (citing Ex. 1002 ¶ 117); *see* Ex. 1001, code (60). Petitioner contends that it would have been obvious to modify Dimov by adopting Han's peelable, two-layer structure, with a reasonable expectation of success. Pet. 40 (citing Ex. 1002 ¶¶ 113–114, 120).

Petitioner does not identify Han as one of the prior art references upon which the Petition bases its challenges. *See* Pet. 8. Additionally, as Patent Owner points out, Petitioner does not actually assert, in the Petition, that Han is prior art to the '408 patent. PO Resp. 15–16. Indeed, we find that the evidence of record does not establish that Han is a prior art publication. Petitioner's own declarant opines that Han's earliest publication date was September 15, 2010 (for the online version). *See* Ex. 1020 ¶ 68. This is after the '408 patent's earliest priority date of July 7, 2010. Ex. 1001, code (60). As Patent Owner correctly points out, Petitioner has not challenged this earliest priority date as applicable to claim 1. PO Resp. 15–16; PO Sur-reply 7 n.2; *see also* Pet. 8, 38 (noting this priority date, without challenging it); Pet. Reply 9 ("Han arguably was published after the earliest potential priority date . . . .").

In its Reply, Petitioner argues that, even if it Dimov itself did not teach the use of a peelable top layer to allow for micropipetting, its argument is based not just on Dimov, but on the background knowledge of a person of ordinary skill in the art. Pet. Reply 8. Citing testimony of Dr. Meinhart,

Petitioner contends that partially bonded PDMS layers and using a micropipette were "well known in the art." *Id.* (citing Ex. 1003, 8:36–40 (describing use of a pipette); Ex. 1007, 9327–28 (describing pipetting as "standard lab practice"); Ex. 1038, 39:7–22; Ex. 1039 ¶¶ 69–78).

Petitioner argues that, "regardless of its prior art status," Han is still "probative of the fact that those skilled in the art would have been motivated to construct a microfluidic device in a way that allowed the top layer of the device to be peeled back to expose microwells with cells that could be selectively aspirated by micropipette." Pet. Reply 9 (citing *Yeda Research v. Mylan Pharm. Inc.*, 906 F.3d 1031, 1041 (Fed. Cir. 2018)).[10]

Patent Owner counters, and we agree, that Petitioner has not shown that the peelable layer in Han's device reflects the background knowledge of a person of ordinary skill in the art at the time of the claimed invention. *See* PO Sur-reply 7–8. As Patent Owner correctly points out, Han describes its microfluidic device as being "*novel*," and Han particularly distinguishes the ability of its device, which allows extraction of embryos directly from a microwell using partially-bonded PDMS layers, from previous devices known in the art, which "had to generate a backward flow to make embryos return to the inlet" so they could be extracted. *Id.* (quoting Ex. 1009, 2853).

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[10] Patent Owner challenges this Reply argument as untimely. *See* Paper 24; Paper 27, No. 12. Petitioner disagrees, citing parts of the record to which it is responsive. Paper 28, No. 12 (citing PO Resp. 15:4–16:2, 36 n.11; Ex. 2012 ¶¶ 84, 137; Dec. 18 n.8). We do not need to decide whether the argument was untimely under 37 CFR § 42.23(b) because, ultimately, we find the argument unpersuasive.

Thus, the evidence of record does not suggest that Han's peelable PDMS layers were known in the art before the date of the claimed invention, and Petitioner has not shown that Han's device reflects the background knowledge of a person of ordinary skill in the art at the relevant time. Consequently, we do not find Petitioner's proposed modification to Dimov to be persuasive.

5. Conclusion as to Grounds Based on Dimov

For the above reasons, we determine that Petitioner has not shown, by a preponderance of the evidence, that Dimov anticipates claim 1 or that claim 1 would have been obvious over Dimov.

Claims 6, 11, 19, 24, 26, 27, and 30 depend, directly or indirectly, from claim 1, and thus incorporate limitation 1E and its requirement of "selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step." *See* Ex. 1001, 32:7–9, 32:23–25, 32:35–38, 32:58–61, 33:6–7, 33:10–14, 33:19–20. Petitioner's analysis of these claims addresses only the added limitations of each dependent claim and does not provide further argument that would remedy the deficiency in Petitioner's analysis comparing claim 1 to Dimov. *See* Pet. 30–34.

Thus, for the reasons given above as to claim 1, Petitioner has not shown that Dimov anticipates claims 6, 11, 19, 24, 26, 27, and 30, or that the claims would have been obvious over Dimov. *See In re Fine*, 837 F.2d 1071, 1076 (Fed. Cir. 1988) ("Dependent claims are nonobvious under section 103 if the independent claims from which they depend are nonobvious.").

D. GROUND BASED ON DIMOV AND KOVAC (GROUND 3)

In Ground 3, Petitioner contends that claims 1, 6, 11, 19, 24, 26, 27, and 30 are unpatentable as obvious over Dimov in view of Kovac. *See* Pet. 40–45.

1. Overview of Kovac

Kovac describes "a microscope-compatible, array-based microfluidic cell sorting architecture centered around passive hydrodynamic trapping of cells and active release using the optical scattering force" (Ex. 1007, 9322) of "a focused infrared laser to levitate cells of interest from their wells into a flow field for collection" (*id.* at 9321). According to Kovac, such "sorting can be a way to select a desired starting population of cells of known characteristics or can be a tool to analyze the results of an experiment and isolate particularly interesting cells for further investigation." *Id.* (footnote omitted).

Figure 1 of Kovac, which illustrates this cell sorting process, is reproduced below:

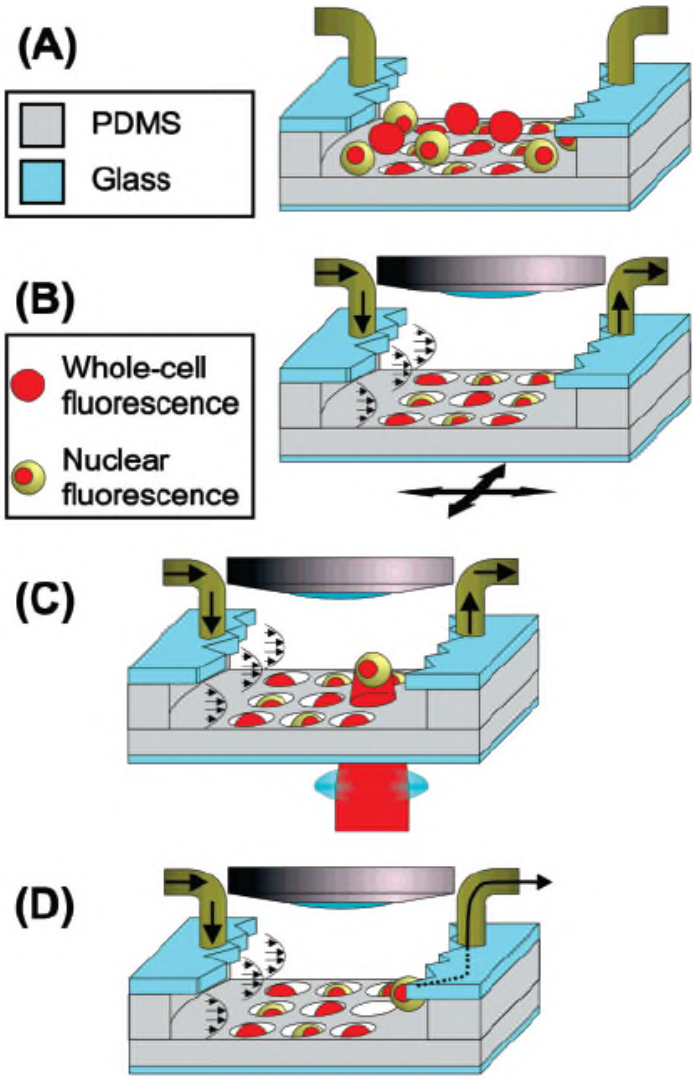

Figure 1, above, depicts a device with two glass layers between which is sandwiched a PDMS layer containing microwells, and above the microwells is a flow field in communication with a fluid inlet and a fluid outlet. *Id.* at 9322. The microwells are 30 µm wide and 35 µm deep, compared to a cell diameter of about 9 µm. *Id.* at 9326. The portion of the PDMS layer below the cells, through which the laser focuses, is about 2 mm thick. *Id.* at 9322–23, 9325.

As depicted in step (A), a fluid containing cells is injected into the device, and when the device is full, the flow is stopped while the cells sediment into the array while the fluid is stationary. Ex. 1007, 9322, 9324.

In step (B), the user pumps fresh media through the device, both forward and backward, and as a result, any cells remaining outside of wells, or which are trapped unstably in the array, flow away from the array and are removed. Ex. 1007, 9322, 9324. During this stage, the fluid outlet goes to an output waste stream. *Id.* at 9323 & Fig. 2.

In step (C), the system scans the entire array using software, and "[a]fter locating cells of interest, we focus an infrared (IR) laser beam onto target cells, levitating the cells into the flow field with the optical scattering force." Ex. 1007, 9322, 9324–25. This levitation "effect is analogous to a beach ball being pushed vertically by a fire hose." *Id.* at 9325. Kovac acknowledges a situation in which two cells could be stably trapped within the same microwell, and demonstrates that it is possible to remove one but not the other. *See id.* at 9326 & Fig. 3.

The width of the laser beam at its focal point is "roughly equal to the cell diameter (~9 μm)." Ex. 1007, 9326. The laser has an output power of up to 150 mW and a wavelength of 980 nm, and is applied for up to about 30 seconds. *Id.* at 9324–25, 9327. Because of potential damage to the cells, the authors view this as "the most extreme parameters we would consider for cell removal." *Id.* at 9327.

According to Kovac, this laser power and wavelength, beam width, and duration "is considerably gentler than parameters in many optical tweezers applications, where the beam is focused to micrometer-sized spots, sometimes at power levels up to ~1 W for longer durations." Ex. 1007, 9327.

Kovac compared these laser parameters to those in other studies and determined that one of the studies, which reported no cell damage using much higher laser power (13.2 W compared to 125 mW in Kovac) and a higher wavelength (1070 nm compared to 980 nm in Kovac) but much shorter exposure time (0.004 seconds compared to 20 seconds in Kovac) and much lower energy density (2.8×10^5 J/cm^2 compared to 4.3×10^6 J/cm^2 in Kovac) was also "an acceptable operating point." Ex. 2004, Table S-1; Ex. 1007.

Finally, in step (D), as the cell is pushed into the flow stream, "[f]luid drag overcomes lateral optical forces, releasing the cell and washing it downstream for fractionation." Ex. 1007, 9322. During this stage, the fluid outlet, with the liberated cell, goes to a reservoir output that can be pierced so that the cell can be removed with a pipette. *Id.* at 9323 & Fig. 2.

2. The Parties' Arguments

For this ground, Petitioner relies on Dimov as teaching limitations 1A–D, as we discuss above in the context of the Dimov-only grounds. Pet. 40; *see supra* Sections III.C.3–.4. In this ground, Petitioner relies on Kovac for teaching limitation 1E. *See* Pet. 40–41.

According to Petitioner, Kovac's method selectively recovers cells by levitating them with a laser so that they "flow to a reservoir of the microfluidic device, where they are removed from the device by micropipette." Pet. 42 (citing Ex. 1007, 9322; Ex. 1002 ¶ 125). In particular, Petitioner argues that an ordinarily skilled artisan would have used a microscope to inspect the trenches of Dimov's device, and then would have "focus[ed] an infrared (IR) laser beam onto target cells from below through the glass substrate and bottom PDMS layer, as described in Kovac, levitating

the cells into the flow field with the optical scattering force." Pet. 43 (citing Ex. 1007, 9322; Ex. 1002 ¶¶ 127–128). Petitioner notes that both the upper and lower layers of Dimov's device are transparent, and the chambers would have been visible in either direction using a microscope. *See* Pet. 44 (citing Ex. 1003, 5:38–39, 6:6–14, 9:13–20, 9:23–27, Figs. 11, 13; Ex. 1002 ¶ 128).

Petitioner contends that, "after levitating a cell of interest from a [chamber] in the Dimov device, the levitated cell could then be flowed down 'a fluid path 103 defined within a substrate 105' to 'an output 130' . . . where it could then be removed from the device by micropipette." Pet. 44 (quoting Ex. 1003, 3:22–26) (citing Ex. 1002 ¶ 128).

Petitioner argues that a person of ordinary skill it the art would have been motivated to modify Dimov's device in this way given "the need to 'select a desired population of cells of known characteristics' or 'isolate particularly interesting cells for further investigation.'" Pet. 43 (quoting Ex. 1007, 9321) (citing Ex. 1002 ¶ 126). According to Petitioner, Kovac teaches that its method "was straightforward, user-friendly, and conceivably automatable," and that it "was simple to implement in a standard microscope with minimal modification." *Id.* (citing Ex. 1007, 9325). Petitioner also argues that a person of ordinary skill in the art "would additionally have been motivated to combine Dimov with Kovac as described above because Dimov expressly discloses removing particles, such as cells, from trenches using '[d]ielectrophoretic' and '[l]aser tweezers' techniques, . . . and Kovac uses similar techniques to accomplish its 'selective levitation.'" Pet. 44 (quoting Ex. 1003, 11:36, 11:38) (citing Ex. 1002 ¶ 130).

Finally, Petitioner contends that there would have been a reasonable expectation of success in combining Dimov with Kovac because "Kovac

discloses that experimental results confirmed that the selective levitation technique yielded the 'expected viability given our optical parameters,' and that '[c]ell retrieval from the device requires a simple pipetting step, which is standard lab practice and unlikely to damage cells." Pet. 45 (alteration in original) (quoting Ex. 1007, 9327–28) (citing Ex. 1007, 9328; Ex. 1002 ¶ 131).

In its Response, Patent Owner first argues, as discussed above in the context of Ground 2 based on Dimov, that if the motivation is to recover cells of interest for later analysis, a person of ordinary skill in the art would not have started with Dimov, which unlike Kovac describes a "lab on a chip" for performing sequential experiments within the same microfluidic device. *See* PO Resp. 17–22; *supra* Section III.C.4(a). According to Patent Owner, to the extent that Kovac provides motivation to sort and isolate cells of interest, using Kovac to provide a motivation to use of Dimov's apparatus shows an improper hindsight bias, as "the Dimov device was simply not designed for selective cell recovery (let alone with the laser technique described in Kovac." PO Resp. 22–23 & n.6 (citing Ex. 2010, 16:9–19, 17:4–9 (Dr. Meinhart agreeing that he did not opine that the challenged claims were obvious over either Kovac alone or over Kovac in view of Dimov)). Patent Owner also contends that Petitioner has not shown "why a [person of ordinary skill in the art] would have focused on selective cell recovery over [the] many other problems" known in the art at the time of the claimed invention. *Id.* at 17.

In its Reply, Petitioner contends that the need for selective cell recovery was known in the art, and that Dimov would have been an appropriate starting point for that endeavor. Pet. Reply 10. According to

Petitioner, the '408 patent admits that "[c]ell recovery is often required to enable functional assays to be performed on the progeny of the input cells, or to select cells of interest for larger scale culture. A method to recover defined clonal populations is therefore a critical requirement for many applications of microfluidic cultures." *Id.* at 10–11 (quoting Ex. 1001, 26:1–6) (citing Ex. 1015, 42 (application as filed)). According to Petitioner, "[s]uch 'applicant admitted prior art' (AAPA) may be used to furnish a motivation to combine." *Id.* at 11.[11] Petitioner also contests that it need not specifically establish why "selective cell recovery," over any other problems, was the motivating reason to modify Dimov's device. *Id.* (citing *KSR*, 550 U.S. at 420).

Next, Patent Owner disagrees with Petitioner that Dimov's mention of laser tweezers as a mixing or removal technique would have led a person of ordinary skill in the art to Kovac's method, because Kovac's laser levitation technique is not laser tweezers, and Kovac distinguishes its technique from laser tweezers. PO Resp. 23 (citing Ex. 2012 ¶¶ 115–118; Ex. 2010, 110:16–23 (Dr. Meinhart agreeing that Kovac distinguishes its laser levitation technique from "traditional optical tweezers")); *see* Ex. 2012 ¶ 117 (Dr. Gale

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[11] Patent Owner contends that Petitioner's reliance on the AAPA is untimely because it first appears in the Reply. Paper 27, No. 2. Petitioner counters that this argument is responsive to statements in the Patent Owner Response that question whether Petitioner has established that a person of ordinary skill in the art would have been motivated to modify Dimov by the problem of selective cell recovery. *See* PO Sur-reply 10 n.4; Paper 28, No. 2 (citing PO Resp. 2:12–18, 16:16–18, 17:5–15). Because Petitioner's AAPA argument is not material to our analysis, as discussed below, we need not determine whether the argument was timely. *See infra* note 14.

testifying that "[t]he laser levitation approach proposed by Kovac does not trap particles, as occurs in laser tweezers, but rather balances particles on a column of laser light, meaning the particles are not held tightly in three dimensions."). According to Patent Owner, Kovac makes modifications to its microwells to achieve its laser levitation technique, such as treating the walls to reduce cell adhesion, that are incompatible with Dimov's goal of allowing for biomimetic experiments that require cell adhesion. PO Resp. 24 (citing Ex. 2012 ¶¶ 109–114).

Petitioner counters that Dimov's reference to laser tweezers need not be so specific as to teach "Kovac's particular mode of laser tweezers" to establish a motivation to combine with Kovac, because Dimov would have encompassed any form of laser tweezers known in the art. Pet. Reply 12 (citing Ex. 1039 ¶¶ 142–148). Relying on Dr. Meinhart's testimony and some of the wording in Kovac, Petitioner disagrees that Kovac's laser levitation method is not a form of laser tweezers. *Id.* (citing Ex. 1039 ¶ 145). Petitioner also argues that "nothing in Dimov requires cell adhesion," so nothing about Kovac's efforts to prevent adhesion during cell recovery would be incompatible with Dimov's goals. *Id.* at 12–13 (citing Ex. 1039 ¶¶ 138–141).

Next, Patent Owner argues that the size of the trenches in Dimov's microfluidic device is incompatible with Kovac's laser levitation technique because the laser in Kovac's device is focused in such a way that, "[a]s the cell is levitated away from the laser focus, the local intensity and lateral gradient force drop due to beam divergence." PO Resp. 24 (alteration in original) (quoting Ex. 1007, 9326). Patent Owner contends that this would be an unsolved problem in Dimov's device because "the cells in Dimov,

according to Petitioner, must be levitated to a height about ten times that in Kovac before they can exit the trenches." *Id.*; *see also id.* at 29.

Patent Owner also argues that, because of the large increase in the distance that cells would have to be levitated in Dimov's much deeper trenches, this "would require prolonged and/or increased exposure to lasers, increasing the risk of damage to the cell." PO Resp. 24–25 (citing Ex. 1007, 9327; Ex. 2004; citing Ex. 2012 ¶¶ 55–56, 102–107, 122–123, 167, 169–170); *see also id.* at 29 n.7 (citing Ex. 2012 ¶¶ 105–107, 170) (arguing that Dr. Meinhart recognized that "avoiding damage to the cell is crucial to success of selective recovery" by framing a reasonable expectation of success in terms of what was "unlikely to damage cells" (citing Ex. 1002 ¶¶ 131, 182)).

Additionally, according to Patent Owner, neither Petitioner nor Dr. Meinhart has explained *how*, given how much larger Dimov's trenches are than Kovac's microwells, a person of ordinary skill in the art "would have gone about focusing an IR laser beam onto target cells to levitate the cell into the flow field of Dimov." PO Resp. 27. Patent Owner argues that, on cross-examination, Dr. Meinhart was not able to say whether or not the "Dimov device's deeper wells would require either prolonged or increased exposure to the IR laser compared to what we see in Kovac" because there are "a lot of factors at play" other than just the height of the trench. *Id.* at 27–28 (quoting Ex. 2010, 168:22–171:1, 171:14–15) (citing Ex. 2012 ¶¶ 92–99).

Patent Owner notes that Dr. Gale agrees with Dr. Meinhart that there are many factors at play, including trench depth, that would determine whether it would be possible to levitate cells from Dimov's trenches using

Kovac's levitation technique. PO Resp. 28 (citing Ex. 2012 ¶¶ 92–99, 102–107, 119–128). Patent Owner also argues that Kovac is silent about how difficult it would be to implement its levitation technique on any other apparatus than its own, but notes that Kovac's model for describing the optical forces involved in levitation are so complex that "intuitive reasoning about behavior" is difficult. *Id.* (quoting Ex. 1007, 9325) (citing Ex. 2010, 100:6–20 (Dr. Meinhart stating that for solving the optical equations for multiple cells, "it's very complicated to do [Kovac's optical calculations] analytically, but you could possibly do it numerically with computer simulations. And [Kovac] does not get into that detail because that's beyond the scope of this work.")). As a result, according to Dr. Gale, a person of ordinary skill in the art would not be able to implement Kovac's levitation technique on a different device, such as Dimov's, by relying on Kovac's disclosure, and would have to conduct new experimental studies. *Id.* (citing Ex. 2012 ¶¶ 102–107, 122–128).

Petitioner counters that Patent Owner "does not substantiate [its] claims" that levitating cells out of Dimov's deeper wells would require prolonged exposure and increase the risk of cell damage. Pet. Reply 13 (citing Ex. 1039 ¶¶ 96–135, ¶¶ 149–151; Ex. 2012 ¶ 106). According to Petitioner, Kovac describes its laser parameters as benign, and the other references it cites use much harsher conditions. *Id.* (citing Ex. 1038, 100:9–101:10; Ex. 1039 ¶¶ 117–132). According to Petitioner, Kovac's data also suggests that the time periods or power levels of Kovac's levitation technique could be extended without causing cell damage, and none of the articles that Kovac cites "concluded that optical trapping techniques—even

harsh ones—should not be used for live-cell applications. *Id.* (citing Ex. 1039 ¶¶ 133–135).[12]

Petitioner also argues that Kovac's teachings are not limited to its specific device, and points out that on cross-examination, Dr. Meinhart stated that so long as devices such as Dimov and Kovac are "submillimeter," they would be comparable. Pet. Reply 14 & n.3 (citing Ex. 1007, 9329; Ex. 2010, 153:10–15; Ex. 1002 ¶¶ 102, 131). Petitioner also points to Dr. Meinhart's cross-examination testimony that there is no need for Petitioner to "identify exact optical parameters and a precise exposure time for removing cells from one of Dimov's trenches, and it would be no bar to obviousness if it were difficult to calculate such parameters, a priori, based on theory due to various factors." *Id.* at 14–15 (citing Ex. 2010, 168:22–171:11). Petitioner ascribes such work to develop the combination of Dimov and Kovac as the ordinary creativity of a person of ordinary skill in the art. *Id.* at 15 (citing *Gen. Elec. Co. v. Raytheon Techs. Corp.*, 983 F.3d 1334, 1352 (Fed. Cir. 2020)). Petitioner argues that Patent Owner has not argued that experimentation would be undue or always required, and contends that, according to Dr. Gale, the experimentation would involve either increasing the exposure time or the laser power, but Petitioner denies that such alterations would result in cell damage. *Id.* at 15 (citing Ex. 2012 ¶ 104).

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[12] Patent Owner contends that Petitioner has improperly incorporated by reference a large portion of Dr. Meinhart's reply declaration without explanation. PO Sur-reply 10–11 n.5. We agree that Petitioner's citations to paragraphs 96–135 and 149–151 (more than 27 pages) are insufficiently explained in the single paragraph on page 13 of Petitioner's Reply. Thus, we do not consider this testimony apart from what Petitioner specifically points out in its Reply. *See* 37 CFR 42.6(a)(3).

Finally, Patent Owner argues that Petitioner has not persuasively shown that, given Dimov's labyrinthine outlet channels that converge from each trench, a person of ordinary skill in the art would have been able to successfully recover for future analysis any cell that has been liberated from Dimov's trench using Kovac's levitation technique. PO Resp. 29–32; *see also supra* Section III.C.3. According to Patent Owner, Dr. Meinhart agreed on cross-examination that in the proposed combination of Dimov and Kovac, the only modification to Dimov's shared output and common waste system would have been to use a "micropipette at the output to extract the cell." *Id.* at 30 (quoting Ex. 2010, 216:9–10) (citing Ex. 2010, 215:2–216:15).

But Patent Owner contends that this proposed modification to Dimov differs from Kovac's device, which includes a "reservoir output" where cells can be collected, separate from the "waste output." PO Resp. 30 n.8 (citing Ex. 1007, 9323–24; Ex. 2010, 67:23–68:25). Patent Owner argues that Petitioner has not explained how a person of ordinary skill in the art would track any cells liberated from the trenches so that they could be recovered somewhere in common waste outputs 130 or 140; for example, the cell would be outside the microscope's field of view. *Id.* at 30–31 (citing Ex. 1003, 4:5–8, 4:20–22, Figs. 1, 2, 4B; Ex. 2012 ¶¶ 63, 97–98, 124–128). According to Patent Owner, a person of ordinary skill in the art "would not have had a reasonable expectation of success following the cell through the device to identify the levitated cell for collection out of the 'common waste.'" *Id.* at 32 (citing Ex. 2012 ¶¶ 30, 97–98, 124–128).

Patent Owner points out that in Dimov's device, each trench shares a common input (120) with seven other trenches, so according to Patent

Owner, flushing a cell out of a trench would involve mixing the trench's outlet stream with fluid from the other neighboring trenches, which adds "a significant amount of fluid to flush the levitated cells through this long path," thus "further disturb[ing] the cell" and possibly mixing the cell with material from other trenches. PO Resp. 31–32 (citing Ex. 2012 ¶¶ 30, 97–98, 124–128). Relying on teachings of Kovac, testimony of Dr. Gale and an acknowledgement of Dr. Meinhart during cross-examination, Patent Owner contends that the convoluted outlet path in Dimov's device would result in "complications such as loss of cells to interior walls, an inability to follow any specific cells of interest, significant dilution, shear stresses, and congestion, none of which support cell recovery." *Id.* at 32 (citing Ex. 1007, 9329 ("[T]he number of contaminating cells collected varies directly with the total time during which target cells are removed."); Ex. 2012 ¶¶ 81, 97–98, 124–128; Ex. 2010, 120:13–121:5 (stating that, according to Kovac, "the longer you spend removing cells, . . . the higher the probability that spurious cells in that solution are collected")).

Petitioner counters that Patent Owner has failed to show that a person of ordinary skill in the art would have been unable to recover levitated cells from Dimov's output stream. Pet. Reply 16 (citing Ex. 1039 ¶¶ 152–156). According to Petitioner, the Petition "never asserted that such monitoring would be required, and Patent Owner does not establish that a [person of ordinary skill in the art] could not accomplish such monitoring, if desired, by using a wider field of view or moving the microscope stage, for example."

Id. (citing Ex. 1039 ¶¶ 155–156).[13] Petitioner also contends that "the challenged claims do not require 100% purity," so the "speculation" that flushing the levitated cell into an outlet could result in mixing with other particles "does not defeat a reasonable expectation of success." *Id.* (citing Ex. 1039 ¶¶ 157–158).

3. Analysis

Like Petitioner's second ground, Petitioner asserts that the motivation to modify Dimov's microfluidic device in view of Kovac's levitation method would have been to allow a user to selectively recover cells of interest for later analysis outside the device. *See* Pet. 43 (identifying the motivation as "the need to 'select a desired population of cells of known characteristics' or 'isolate particularly interesting cells for further investigation'" (quoting Ex. 1007, 9321)). For the reasons below, we find that Petitioner has not proven this motivation.[14] Thus, Petitioner has not shown, by a preponderance of the evidence, that claim 1 would have been obvious over the combination of Dimov and Kovac.

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[13] Patent Owner contends that this argument is untimely because Petitioner first raised it in the Reply. Paper 27, No. 4; *see* Paper 28, No. 4 (Petitioner's response). Because Petitioner's assertion is not material to our decision, we do not need to decide whether it is untimely.

[14] As discussed above, Petitioner alleges that the '408 patent includes applicant admitted prior art (AAPA) establishing a known general motivation to selectively recover cells. *See supra* note 11; Ex. 1001, 26:1–6. We need not decide whether this passage is AAPA because, even if it were, it does not establish a motivation to combine Dimov and Kovac, specifically, to achieve the asserted goal of selective cell recovery.

First, we agree with Petitioner that its burden, under *KSR*, does not require it to show why selective cell recovery, over any other known problem in the art, would have been the motivating reason to modify Dimov's device. *See* Pet. Reply 11. *See KSR*, 550 U.S. at 420 ("[A]ny need or problem known in the field of endeavor at the time of invention and addressed by the patent can provide a reason for combining the elements in the manner claimed."). But "[a]lthough the *KSR* test is flexible, the Board 'must still be careful not to allow hindsight reconstruction of references.'" *Trivascular, Inc. v. Samuels*, 812 F.3d 1056, 1066 (Fed. Cir. 2016) (quoting *Kinetic Concepts, Inc. v. Smith & Nephew, Inc.*, 688 F.3d 1342, 1368 (Fed. Cir. 2012)). Petitioner must, at least, show that a person of ordinary skill in the art would have had reason to select Dimov's device as a suitable starting point to achieve what Petitioner contends is the motivating objective. *See Unigene Labs., Inc. v. Apotex, Inc.*, 655 F.3d 1352, 1360 (Fed. Cir. 2011) ("[O]bviousness requires the . . . showing that a person of ordinary skill at the time of the invention would have selected and combined those prior art elements in the normal course of research and development to yield the claimed invention.").

Second, we agree with Patent Owner that Petitioner has failed to meet its burden to show that a person of ordinary skill in the art would have selected Dimov's microfluidic device for use with Kovac's cell levitation method. That Dimov briefly mentions "[l]aser tweezers" as a possible mixing or removal technique (Ex. 1003, 11:38) is not persuasive evidence that a person of ordinary skill in the art would have selected Kovac's *specific* laser levitation method for use in Dimov's device to recover cells of interest for further analysis.

The evidence shows that the ability to recover useful cells, using laser-based manipulation, is highly dependent on the laser parameters, including power, exposure time, and the shape of the laser focus. *See, e.g.*, Ex. 1007, 9325–26; Ex. 2012 ¶ 104. For example, Kovac teaches that "[a]s the cell is levitated away from the laser focus, the local intensity and lateral gradient force drop due to beam divergence." Ex. 1007, 9326; Ex. 2012 ¶¶ 50, 104. Although Kovac uses a beam divergence such that, despite this drop, maintains cells within the beam while levitating them into the flow stream above Kovac's shallow 35 μm microwells (*see* Ex. 1007, 9326), Petitioner's proposed combination would require focusing the laser beam such that it can raise a cell nearly an order of magnitude higher than in Kovac, and Petitioner has not shown how a person of ordinary skill in the art would have obtained laser parameters suitable for raising the cell that far.

Similarly, Petitioner has not shown how a person of ordinary skill in the art would have addressed the problem, discussed in Kovac, that under certain combinations of power, spot size, and exposure time, there is likely to be unacceptable cell damage that would motivate against using Kovac's method in Dimov's device to recover cells that are still usable for further analysis as per Petitioner's alleged motivation to combine. *See* Ex. 1007, 9326–28; Ex. 2004, Table S-1. Kovac teaches that "the most extreme parameters [the authors] would consider for cell removal" in its 35 μm microwells are 150 mW applied to the cells for 30 seconds. Ex. 1007, 9327. But these parameters are designed for Kovac's device, which uses a microwell that is nearly an order or magnitude narrower and shallower than Dimov's trench.

Kovac teaches that, other than knowing that "scattering force is linearly proportional to power," further intuitive reasoning about scattering behavior used in designing a laser levitation column is "difficult without focusing on a specific set of optical parameters." Ex. 1007, 9325. Both Drs. Meinhart and Gale agree that there are "a lot of factors at play" when designing a laser column that could successfully levitate a cell into a flow stream without causing unacceptable cell damage. *See* Ex. 2010, 168:22–171:11; Ex. 2012 ¶¶ 92–99, 102–107, 119–128.

Yet, the uncontested level of ordinary skill in the art, which Petitioner proposed and we have adopted for this decision, involves only a bachelor's degree and as little as three years of experience constructing and using microfluidic devices for cell culture and analysis. *See supra* Section III.A. Beyond that level of expertise, this articulation does not require any specialized knowledge of the use of lasers to manipulate living cells. We find that Petitioner has not shown, by a preponderance of the evidence, that such a person of ordinary skill in the art would have had the background knowledge and experience needed for manipulating the many "factors at play" to successfully adapt Kovac's method for use in Dimov's much deeper trenches. Nor has Petitioner demonstrated that it would have even been feasible to selectively recover cells from Dimov's deep trenches that are suitable for future analysis outside Dimov's device.[15]

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[15] Because we find that Petitioner has not persuasively proven its purported motivation to combine, we need not address the question of whether there would have been a reasonable expectation of success in modifying Dimov to achieve limitation 1E as claimed (which does not explicitly require the recovery of viable cells). *See Intelligent Bio-Sys., Inc. v. Illumina Cambridge*

We also find that Petitioner has not shown that a person of ordinary skill in the art would have considered it feasible to recover liberated cells from Dimov's shared waste stream, given the long path from each trench to the waste stream and the fact that potentially hundreds of other trenches share the same outlet. *See supra* Section III.C.3 (discussing challenges that would be involved in recovering a specific cell from Dimov's device). Dimov's outlet is substantially different from that of Kovac, which has a single outlet for the array and a reservoir output, separate from the waste stream, where individual liberated cells travel to await recovery with a micropipette. *See* Ex. 1007, 9322–23 & Figs. 1, 2A. Given such differences and the lack of any such disclosure in Dimov itself, we determine that Petitioner has not explained, with enough specificity, how a person of ordinary skill in the art would have located and recovered a liberated cell from Dimov's common waste stream. *See Personal Web Techs., LLC v. Apple, Inc.*, 848 F.3d 987, 994 (Fed. Cir. 2017) ("[A] clear, evidence-supported account of the contemplated workings of the combination is a prerequisite to adequately explaining and supporting a conclusion that a relevant skilled artisan would have been motivated to make the combination and reasonably expect success in doing so.").

For the above reasons, we determine that Petitioner has not shown, by a preponderance of the evidence, that claim 1 would have been obvious over Dimov in view of Kovac.

Ltd., 821 F.3d 1359, 1367–68 (Fed. Cir. 2016) (although reasonable expectation of success considers the invention as claimed, a skilled artisan's ability to achieve the intended outcome is relevant to the motivation to combine).

Beyond its arguments in the context of anticipation, Petitioner does not address the specific additional limitations of dependent claims 6, 11, 19, 24, 26, 27, and 30. *See* Pet. 40–45. Thus, for the reasons given above as to claim 1, Petitioner has not shown sufficiently that claims 6, 11, 19, 24, 26, 27, and 30 would have been obvious over Dimov in view of Kovac. *See Fine*, 837 F.2d at 1076.

E. GROUND BASED ON PARK (GROUND 4)

In Ground 4, Petitioner contends that claims 1, 11, 16, 24, 26, 27, and 30 are unpatentable under 35 U.S.C. § 103(a) as obvious over Park. *See* Pet. 45–61. As we discuss below, we determine that Petitioner's arguments are not persuasive.

1. Overview of Park

Park is a journal article describing "a flow method that enables single cell trapping in microwells with dimensions of 50 μm, a size sufficient to allow attachment and division of captured cells." Ex. 1006, 263. According to Park, prior studies had described methods for trapping multiple cells in each well, but "[s]ingle cell trapping is necessary to allow identification of differing cell phenotypes within a population of cells" and "enables observation of the direct descendants of single cells cultured under controlled biological conditions . . . , the analysis of intracellular compounds . . . , and the measurement of electrical functionality of cells." *Id.*

Thus, Park designed a triangular microwell array configured so that, "[o]nce a cell is captured, the cell presence in the microwell changes the flow pattern, thereby preventing trapping of other cells." Ex. 1006, 263. This is illustrated in Figures 1a–c of Park, reproduced below:

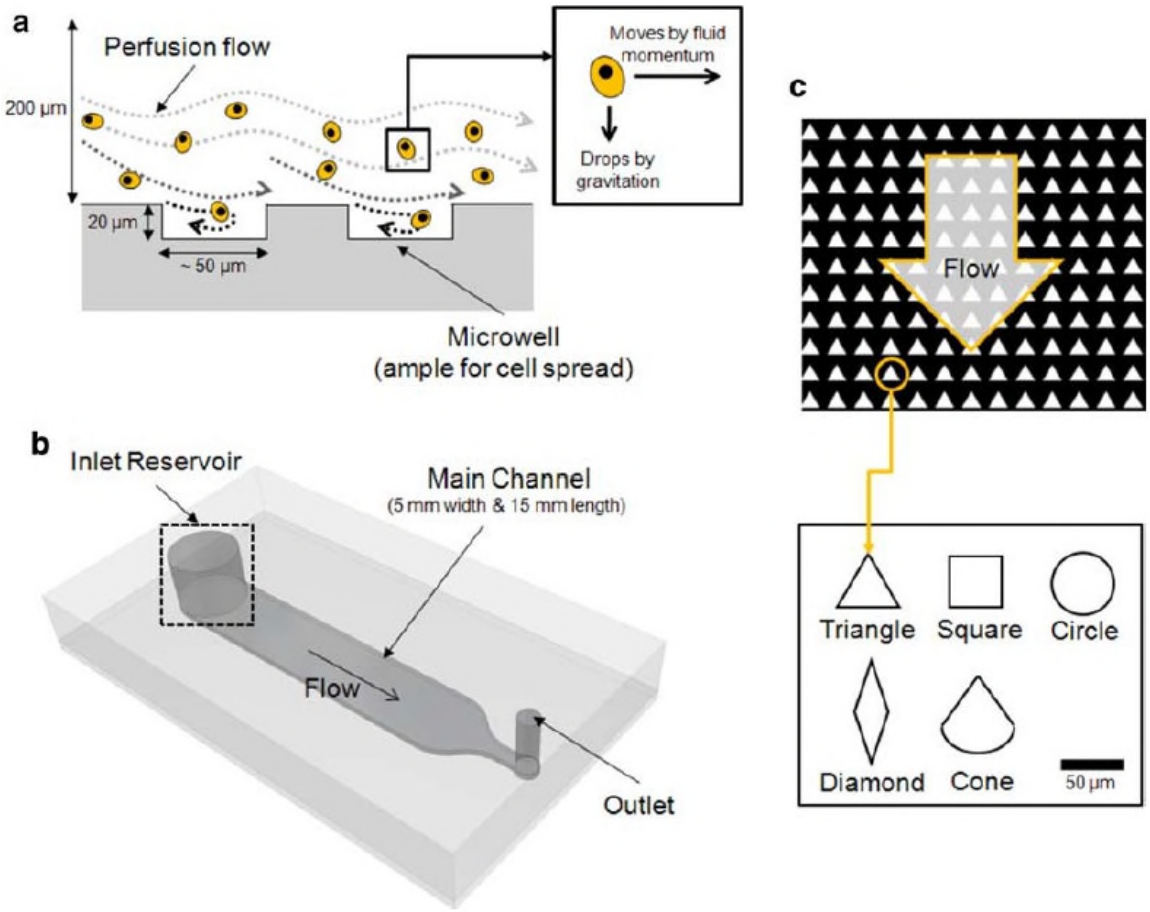

Figure 1b, above, shows the overall system, which includes an "inlet reservoir where the cell suspension is introduced, [a] main channel, microwells (not shown in the figure) patterned on the bottom surface of the main channel, and the outlet which is connected to a pulling syringe pump." Ex. 1006, 264.

Figure 1a, above, is a cross-sectional view of the microwells (20 µm deep and 50 µm wide) and the main channel (200 µm deep). Ex. 1006, 264. "During the perfusion of cell suspension in the channel, the cells sink gradually due to gravity, and some cells are caught in microwells while others pass and are carried away by flow." *Id.* at 264. According to Park, this is because, "[g]enerally, cells are 2–5% heavier than culture medium." *Id.* at 265. "Depending on the velocity of the cell and its position relative to the

microwells, a cell may approach a threshold slow speed at which point gravity shifts the cell into a lower streamline that leads into a recirculation zone in the microwell." *Id.*

Figure 1c, above, shows various microwell shapes that the authors tested in simulation, and shows the orientation of triangular microwells compared to the flow direction. *Id.* The authors "determined the equilateral triangle to be the best well shape because it had the strongest recirculation pattern in the microwell from 3D simulations." Ex. 1006, 265–66.

After flowing the cell suspension across the array, the user can "flush the system . . . to remove excess cells" at a flow rate that "is fast enough to wash away untrapped cells, but slow enough not to dislodge trapped cells." Ex. 1006, 268.

2. Uncontested Limitations 1A–D

Petitioner contends that Park discloses the preamble and limitations 1A–D, and points to passages within Park describing retaining a cell in a chamber, perfusing the cell with fluid while the cell is retained in the chamber, and culturing the cell within the chamber while monitoring a response. Pet. 45–57 (citing Ex. 1006, 264–68, Figs. 1–3; Ex. 1002 ¶¶ 132–159).

Patent Owner does not dispute Petitioner's allegation that Park discloses limitations 1A–D. *See generally* PO Resp. We need not address Petitioner's allegations regarding the preamble or limitations 1A–D because we determine, below, that Park does not disclose or teach limitation 1E.

3. Limitation 1E

For the selective recovery step of limitation 1E, Petitioner argues that because Park discloses culturing cells and monitoring responses in the triangular microwells, "[i]t would have been obvious to a [person of ordinary skill in the art] to make use of this monitoring by selectively removing one or more cells from an individual microwell based on a response—e.g., a long-term cell response or an instantaneous cell reaction—observed through such monitoring." Pet. 57 (citing Ex. 1002 ¶ 160).

To accomplish this, Petitioner contends that a skilled artisan "would have found it obvious to construct the device of Park in a known way that would permit the top layer of the device to be removed or peeled back so a microwell could be accessed directly by micropipette." Pet. 57 (citing Ex. 1002 ¶ 161). Petitioner contends that Park's device has two layers, and although Park does not disclose what materials the layers are made of, "it would have been obvious to a [person of ordinary skill in the art] to make such layers from PDMS." Pet. 57 (citing Ex. 1002 ¶ 162).

Because "Park does not state that the two layers of its device are bonded together," Petitioner contends that this "suggest[s] that the top layer may actually be removable," but even if Park's top layer is not removable, according to Petitioner, an ordinarily skilled artisan "would have found it obvious to construct the device so that it would be removable or to partially bond the top layer to the bottom layer so the top layer could be peeled back to expose the microwell array." Pet. 58 (citing Ex. 1002 ¶¶ 163–164).

As with its ground asserting obviousness based on Dimov, Petitioner relies on Han to support its proposed modification of Park's device. *See*

Pet. 58 (citing Ex. 1009, 2849; Ex. 1002 ¶¶ 164–165); *supra* Section III.C.4(b).

In its Response, Patent Owner contends that nothing in Park suggests selectively recovering cells that had been trapped in its microwells based on a monitored response, or that Park's device would have been suitable for such selective recovery of cells. *See* PO Resp. 33–37. In particular, Patent Owner contends that Park's device was not designed for selective recovery, and focuses instead on ensuring that the microwells trap single cells yet still have space for subsequent attachment and division of captured cells within the microwells. *Id.* at 33–36 & n.10 (citing Ex. 1006, 263–65, 267, 268, Fig. 3c; Ex. 2010, 123:3–6; Ex. 2012 ¶¶ 46–47, 130–136).

According to Patent Owner, Park's device did not "allow external access to the chambers," so to allow for selective access to cells, a user would have had to redesign Park's device in a way that has no basis within Park's teachings. PO Resp. 36–37 (citing Ex. 1006, 265, 268 ("The main limitations of our system are . . . that a cover is required to create the channel, restricting access to the microwells during cell seeding . . . ."); Ex. 2012 ¶¶ 136–138). Thus, Patent Owner contends that Petitioner's argument suffers from improper hindsight bias. *Id.* at 37 (citing *Cheese Sys.*, 725 F.3d at 1352). Patent Owner also contends that Petitioner's reliance on Han for this teaching is improper for the same reasons it is improper in the context of Dimov's disclosure. *Id.* at 36 n.11.

In its Reply, Petitioner counters that Patent Owner does not dispute that Park (1) is relevant prior art, (2) discloses limitations 1A–D, (3) is successful in culturing cells within the microwells, and (4) worked for its intended purpose. Pet. Reply 17. Petitioner also disputes Patent Owner's

contention that Park discloses a closed system to which there is no direct access to microwells, because Park "implies the opposite when it discloses that the top layer or cover of its device merely 'fits over' the bottom layer, and notes that this 'cover is required to create the channel, restricting access to the microwells *during cell seeding*." *Id.* at 18 (quoting Ex. 1006, 265, 268). Petitioner contends that "[t]he qualification 'during cell seeding' would have suggested to a [person of ordinary skill in the art] that there is, in fact, access to the microwells at times other than during cell seeding." *Id.* (citing Ex. 1039 ¶¶ 164–173).[16] According to Petitioner, a skilled artisan would have displaced the cover to aspirate cells from a microwell via a micropipette. *Id.* (citing Ex. 1002 ¶¶ 160–165 (relying on Dimov and Han)).

We agree with Patent Owner that Petitioner has not pointed to anything in Park that teaches selectively recovering cells. Petitioner's attempt to infer such a motivation from Park's disclosure relies on improper hindsight bias. *See* PO Sur-reply 17–18. In particular, Park itself does not appear to motivate making the top layer removable and aspirating cells from microwells using a micropipette. At most, Petitioner has only shown why an ordinarily skilled artisan *could have* modified Park, not that there would have been actual motivation to make the proposed modifications. *See Belden Inc. v. Berk-Tek LLC*, 805 F.3d 1064, 1073 (Fed. Cir. 2015) ("obviousness concerns whether a skilled artisan not only *could have made* but *would have*

[16] Patent Owner contends that this argument is untimely because it first appears in the Reply. Paper 27 No. 8; see Paper 28, No. 8 (Petitioner's response). Because we ultimately find this argument unpersuasive, we need not decide whether it is untimely.

been motivated to make the combinations or modifications of prior art to arrive at the claimed invention").

As to Petitioner's reliance on Han as evidence that it was known in the art to make a microfluidic device peelable to allow access by a micropipette, we find this reliance insufficient for the reasons we discuss above in the context of the obviousness ground based on Dimov. *See supra* Section III.C.4(b); *see also* PO Resp. 18–20 (noting further distinctions between Park and Han).

For the above reasons, we determine that Petitioner has not shown, by a preponderance of the evidence, that claim 1 would have been obvious over Park. Petitioner's arguments as to claims 6, 11, 16, 24, 26, 27, and 30 address only the added limitations of each dependent claim and do not provide further argument that would remedy the deficiency as to limitation 1E. *See* Pet. 59–61. Thus, Petitioner has not shown that these claims would have been obvious over Park. *See Fine*, 837 F.2d at 1076.

F. GROUND BASED ON PARK AND KOVAC (GROUND 5)

In its Ground 5, Petitioner contends that claims 1, 11, 16, 24, 26, 27, and 30 are unpatentable as obvious over Park in view of Kovac. *See* Pet. 62–63. For the reasons below, we determine that Petitioner has not proven unpatentability based on this ground by a preponderance of the evidence.

1. The Parties' Arguments

For this ground, Petitioner relies on Park for teaching limitations 1A–D, as discussed above in the context of the Park-only ground (Ground 4), and relies on Kovac for teaching limitation 1E. *See* Pet. 62. According to Petitioner, a person of ordinary skill in the art "would have been motivated

to add the 'selective levitation' technique of Kovac, as described in [Petitioner's third ground], to the methods disclosed in Park." *Id.*

In particular, Petitioner contends that an ordinarily skilled artisan would have used a microscope to inspect Park's microwells, and then would have "focus[ed] an infrared (IR) laser beam onto target cells from below, as described in Kovac, levitating the cells into the flow field with the optical scattering force." Pet. 62 (citing Ex. 1007, 9322; Ex. 1002 ¶ 180). Petitioner contends that it would have been "obvious to use a transparent or semi-transparent material, such as PDMS, to construct the top layer of the Park device so as to permit visual observation of microwells from above." Pet. 63 (citing Ex. 1002 ¶ 181). After levitating a cell, according to Petitioner, an ordinarily skilled artisan would then allow the cell to "flow[] down the 'main channel' to the existing 'outlet,' 'which is connected to a pulling syringe pump' to evacuate the cell from the device." *Id.* (quoting Ex. 1006, 264) (citing Ex. 1002 ¶ 181).

Petitioner contends that Kovac's microwells, 30 μm in diameter and 35 μm deep, are similar in size to Park's microwells, 50 μm per side and 20 μm deep. Pet. 63 (citing Ex. 1007, 9326; Ex. 1006, 266). Petitioner also contends that Kovac teaches that its method would yield viable cells, and only requires a simple pipetting step to recover the cells. Pet. 63 (citing Ex. 1007, 9327–28).

In its Response, Patent Owner first argues that because Park's device was not designed to allow for selective recovery of cells, "there would have been no reason for a [person of ordinary skill in the art] to have started with the Park device." PO Resp. 38–39 (citing PO Resp. 33–37 (similar argument in the context of the ground based on Park alone); Ex. 2012 ¶¶ 139–159). As

with Dimov, Patent Owner contends that Park's device "was designed to *trap* cells for culturing *within* the microwells of the device." *Id.* at 39 (citing Ex. 2012 ¶¶ 41–46, 139–159). It is "a closed system having a single inlet and outlet" and "there was no way for a [person of ordinary skill in the art] to access the culturing cells without first disassembling the device and losing the flow through the device, which would allow any cells to end up back in the device." *Id.* at 39–40 (citing Ex. 1006, 264–67; Ex. 2010, 142:6–8, 142:19–23; Ex. 2012 ¶¶ 44, 151–159); *see also id.* at 44 (citing Ex. 1006, 264–67; Ex. 2010, 142:6–8, 19–23; Ex. 2012 ¶¶ 52, 155–158) (noting that, unlike Kovac, Park has no outlet reservoir for recovering specific cells); *see also id.* at 53–54 (citing Ex. 2010, 141:14–16, 146:25–147:19, 150:18–151:2; Ex. 2012 ¶¶ 42, 155, 168) (arguing that Petitioner has not explained how a person of ordinary skill in the art would have modified Park's device to allow for recovery of a specific liberated cell, other than disconnecting the syringe pump and thus disrupting the flow).

Next, Patent Owner argues that Petitioner has failed to show a motivation to combine Park with Kovac because, whereas Park's device uses circulation patterns caused by fluid flow to trap cells within a well, "Kovac uses the lack of flow to trap cells, and does not discuss replication of cells in the wells" and "makes efforts to prevent the cells in its wells from becoming adhesive (by using suspension and pre-treating the wells), which is the opposite of Park's goal." PO Resp. 43 (citing Ex. 2010, 202:3–11; Ex. 2012 ¶¶ 45–46, 53, 147–149, 151–159); *see also id.* at 44 (citing Ex. 1006, 264–67; Ex. 2010, 83:23–84:5, 138:2–14, 142:6–8, 19–23; Ex. 2012 ¶¶ 42–46). Patent Owner argues that Park's device is designed so that cells will attach to each other and to the wells during culturing, and Kovac contains no

teachings about using its levitation method under these very different circumstances. *Id.* at 49–50 (citing Ex. 1006, 267, Fig. 3c; Ex. 2002, 58:23–26; Ex. 2010, 117:12–119:7; Ex. 2012 ¶¶ 53–56, 161, 165).[17]

According to Patent Owner, "the triangular shaped microwells of Park were specifically designed to retain cells during flow," yet in Kovac's device, fluid flow is necessary for recovering any cells levitated by the laser. PO Resp. 47 (citing Ex. 1007, 9322; Ex. 2010, 89:8–11; Ex. 2012 ¶¶ 162–166). Thus, Patent Owner argues that in Petitioner's proposed combination, "the dynamic flow of fluid through the microwells in Park would suppress the levitation force of the Kovac laser, hampering recovery." *Id.* (citing Ex. 2012 ¶¶ 162–166). According to Patent Owner, counteracting this downward force "would require prolonged and/or increased exposure to lasers, increasing the risk of damage to the cell." *Id.* at 48 (citing Ex. 2012 ¶¶ 55–56, 102–107, 122–123, 167, 169–170).

Next, Patent Owner points to other factors that would have complicated the proposed combination, such as that Park's recirculation pattern drags cells not just downward but to an upstream corner where Stokes drag wall effects would have led to longer removal times from the microwell and failure of cell removal. PO Resp. 48 (citing Ex. 1006, 266–67, Fig. 2c; Ex. 1007, 9326 ("[N]onspecific surface interactions appear to be

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[17] Although Patent Owner acknowledges that Kovac discusses removing a single cell from a well with multiple cells, Patent Owner contends that this only relates to a doubly loaded cell in which the cells are not the result of replication due to culturing and are not adhered to each other. PO Resp. 43 n.15 (citing Ex. 1007, 9326; Ex. 2010, 221:4–222:4 (Dr. Meinhart agreeing that this example in Kovac is for a single cell in a doubly loaded well); Ex. 2012 ¶¶ 148–149, 157).

the primary reason that cell removal fails."); Ex. 2010, 115:5–117:11, 112:22–113:23, 139:5–9; Ex. 2012 ¶¶ 162–166).

Patent Owner also argues that Dr. Meinhart acknowledged that a person of ordinary skill in the art would have needed to modify the laser parameters, such as by using a different lens, to use Kovac's levitation method in Park's device. PO Resp. 50 (citing Ex. 2010, 132:9–136:12). And according to Patent Owner, Dr. Meinhart recognized that there were "a lot of factors at play" that would affect the levitation, such as "cell size, whether cells are conglomerated together, . . . wall interactions . . . recirculation patter[n]s, [and] hydrodynamic drag," such that "[i]t'd be difficult to determine how much time would be necessary to be exposed." *Id.* at 50–51 (first, second, and fourth alterations in original) (quoting Ex. 2010, 168:22–171:7). Patent Owner reiterates its arguments, discussed above in the context of combining Dimov with Kovac, that Kovac's levitation technique involves complicated factors for which intuitive reasoning is difficult, so a person of ordinary skill in the art "would need to experiment to figure out if a cell could be levitated out of a well." *Id.* at 51 (citing Ex. 1007, 9327; Ex. 2010, 100:6–20; Ex. 2012 ¶¶ 96, 120, 160–166).

Patent Owner also contends that if Kovac's levitation method were used in Park's device, any liberated cell would "need to pass over the array of other microwells before reaching the common outlet," which according to Park are only 62% filled, leaving "approximately 40% of the microwells open and in which the levitated cell could be recaptured" by recirculation patterns caused by the flow necessary to extract a cell. PO Resp. 52–53 (citing Ex. 1006, 263–64; Ex. 1007, 9329; Ex. 2010, 120:13–121:5, 141:4–13; Ex. 2012 ¶¶ 167–170).

In its Reply, Petitioner disagrees that it would have been difficult for a person of ordinary skill in the art to use a different lens or otherwise adjust the laser parameters to adapt Kovac's levitation method for use in Park's device. Pet. Reply 23–24. Petitioner only acknowledges that there would be difficulty to say, "a priori, whether levitation out of deeper wells would take longer," and contends that Kovac only stated that intuitive reasoning about its optical equations would be difficult to predict "without focusing on a specific set of optical parameters." *Id.* at 23 (emphasis omitted) (quoting Ex. 1007, 9325). According to Petitioner, Dr. Meinhart testified that it would have been possible to adapt Kovac's technique "without actually calculating numbers from the equation." *Id.* at 23–24 (quoting Ex. 2010, 100:2–5) (citing Ex. 1039 ¶ 149).

Petitioner also provides a number of further rationales for how a person of ordinary skill in the art would have modified Park's device to work with Kovac's levitation method.[18] To address Patent Owner's argument that Petitioner has not explained how to recover specific cells from Park's device, Petitioner contends that an ordinarily skilled artisan would have sucked a liberated cell "directly into the pump's syringe" before disconnecting it. Pet. Reply 20 (citing Ex. 1002 ¶ 181, Ex. 1039 ¶¶ 184–188).

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[18] Patent Owner contends that these arguments are untimely, and we agree at least as to certain of the arguments. *See* Paper 27, Nos. 6, 9, 10; *see* Paper 28, Nos. 6, 9, 10 (Petitioner's responses). The new arguments introduce new theories as to how to combine Park and Kovac, including additional steps or new modifications necessary for a successful combination, which could have been presented in the Petition but were not. Nevertheless, as we discuss below, we find these arguments unpersuasive.

Petitioner also contends, for the first time, that a person of ordinary skill in the art would have addressed the problem of Park's downward-forcing circulation patterns[19] by starting with flow turned off and then turning on flow "after levitation begins, including after the cell has been pushed up out of a well high enough to be clear of recirculating flow lines created once it is turned on." Pet. Reply 19–20 (citing Ex. 1039 ¶¶ 175–177); *see also id.* at 22 (citing Ex. 1039 ¶ 193).

Alternatively, Petitioner asserts that an ordinarily skilled artisan would have adjusted the size of Kovac's laser column to cover Park's entire triangular microwells, so that a cell would have no place to be pushed out of the laser column by the circulating flow. *See* Pet. Reply 21–22 (citing Ex. 1039 ¶¶ 190–196). Petitioner asserts that, because this wider beam would have lower power density, this would "*decrease* the risk of damage to the cell." *Id.* at 22 (citing Ex. 1039 ¶¶ 193, 205–206).

On the issue of whether cell attachment associated with culturing of the cells in Park's device would hamper the ability to levitate the cells, Petitioner acknowledges that Park "states that its wells are of 'a size sufficient to allow attachment and division of captured cells.'" Pet. Reply 24 (quoting Ex. 1006, 263). But Petitioner asserts that "Park nowhere teaches

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[19] Petitioner contends that "Patent Owner fails to explain how resisting lateral movement would affect selective recovery by levitation, and does not establish that any resistance to vertical movement would be problematic either." Pet. Reply 24 (citing Ex. 1039 ¶¶ 190–196). This improperly shifts the burden of persuasion onto Patent Owner. *See Dynamic Drinkware, LLC v. National Graphics, Inc.*, 800 F.3d 1375, 1379–81 (Fed. Cir. 2015) (a petitioner has the burden of persuasion in an *inter partes* review, which does not shift to the patent owner).

that its device can be used only with cells that attach within wells, let alone those that remain attached." *Id.* (citing Ex. 1039 ¶¶ 180–183, 189).

As to the issue that cells liberated in Park's device would have to pass over other empty wells that might recapture the cells, Petitioner responds that a person of ordinary skill in the art could simply repeat the process at the empty well that the cell fell into, which Petitioner contends would not require the use of extraordinary skill or creativity. Pet. Reply 25. Petitioner also argues that "the probability that a levitated cell will be recaptured is very low," which Dr. Meinhart calculates as "about 13.8%." *Id.* (citing Ex. 1006, 268; Ex. 1039 ¶¶ 197–204).

In its Sur-reply, Patent Owner contends that "there are no experiments performed in Park where the flow is turned on and off after cells are permitted to culture." PO Sur-reply 22 (citing Ex. 2013, 358:25–359:9). Thus, Patent Owner contends that it is unknown whether "this would have resulted in additional cells dislodging when the flow was turned on and off for each attempt at selective cell recovery," and "it is unknown how this may have affected the technique or cell health, including overheating due to the lack of cell medium exchange from the flow of the device." *Id.* Patent Owner also contends that "Petitioner and Dr. Meinhart provide no mechanism for a [person of ordinary skill in the art] to have been able to determine when the cell was 'high enough to be clear of recirculating flow.'" *Id.*

Similarly, Patent Owner contends that Petitioner's proposed modification of disconnecting Park's syringe after recovering a cell in the syringe also "would have required a [person of ordinary skill in the art] to turn on and off the flow of Park . . . , which would increase the number of

non-target cells dislodged." PO Sur-reply 24 (citing Ex. 1039 ¶ 188). And Patent Owner contends that Petitioner "does not explain how a [person of ordinary skill in the art] would have been able to determine when the cell enters the pump so it could be disengaged for recovery." *Id.* (citing Ex. 1038, 111:18–113:6).

Patent Owner also argues that Kovac teaches that the width of its laser column was "specifically selected to levitate a single cell from its microwell 'without perturbing cells in neighboring wells.'" PO Sur-reply 23 (quoting Ex. 1007, 9325–26 ("We . . . demonstrated the feasibility of selectively levitating a single . . . cell from a microwell without perturbing cells in neighboring wells. We focused the beam so that the beam waist was roughly equal to the cell diameter (~9 μm).")). But according to Patent Owner, increasing the width of the laser column to cover Park's entire microwell "would have disturbed cells in neighboring wells." *Id.* Patent Owner also contends that if spot size increases to cover Park's microwells (which are about 50 μm per side),[20] Dr. Meinhart acknowledges that this "will decrease the lateral gradient force," but "Dr. Meinhart . . . did not consider how this reduction in force would affect the laser's ability to levitate a cell in Park's well successfully (especially with cultured cells through Park's recirculation pattern)." *Id.* (quoting Ex. 2013, 344:18–345:5).

Finally, Patent Owner disagrees with the methodology of Dr. Meinhart's estimate of a 13.8% probability that cells would be recaptured in

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[20] Patent Owner contends that "Kovac already used a spot size that was 'larger than those typically used.'" PO Sur-reply 24 (quoting Ex. 1007, 9325).

Park's microwells while passing over the array. PO Sur-reply 24–25. Even if the number is correct, Patent Owner contends, Kovac also discloses a loss of 18–28% of cells with its technique, so compounding the two losses from Park and Kovac "would have resulted in the loss of at least a third of the first cells that [were] successfully levitated." *Id.* at 25 & n.11.

2. Analysis

Considering the evidence as a whole, we find that Petitioner has not met its burden to show obviousness based on the combination of Park and Kovac. As discussed above in the context of the combination of Dimov and Kovac, the evidence indicates that Kovac's laser levitation method is highly dependent on the laser parameters including power, exposure time, and the shape of the laser focus. *See supra* Section III.D.3. Petitioner's proposed modifications to Kovac's levitation method, such as substantially increasing the focused spot size (which was already "larger than those typically used in optical tweezers applications," Ex. 1007, 9325), would have required substantial experimentation. Yet, as discussed above in the context of the Dimov–Kovac combination, the uncontested level of ordinary skill in the art only requires a bachelor's degree and 3–5 years of general experience constructing and using microfluidic devices for cell culture analysis. *See id.* Petitioner has not shown that such a person would have had sufficient background in using lasers to manipulate living cells that they could have adapted Kovac's levitation method for use in Park's device without additional inventive steps.

One particular complication would have been that Park's device is designed to create recirculating flow patterns whenever there is flow through the device (as is required in Kovac's method, *see* Ex. 1007, 9322, 9324–25;

Ex. 2012 ¶ 162), and we credit Dr. Gale's testimony that these would have worked against any laser levitation. *See* Ex. 2012 ¶¶ 163–164; Ex. 1006, 266, Fig. 2a. We also credit Dr. Gale's testimony that wall effects, or adhesion between cells and walls or other cells, would likely occur after culturing the cells in Park's microwells,[21] and would have been complicating factors for which neither Park nor Kovac provide solutions.[22] *See* Ex. 2012 ¶¶ 161, 165.

We do not find persuasive Petitioner's new argument in the Reply that, to address this complication, a person of ordinary skill in the art would have begun Kovac's levitation procedure with fluid flow turned off, and then turned the fluid on at some point during the levitation where the flow pulling the cell into the fluid stream would be greater than the flow pulling it back into the microwell. Pet. Reply 19–20. Petitioner does not point to anything

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[21] Kovac does not appear to discuss the problems that would arise from levitating cells after culturing them in a microwell. Although Dr. Meinhart notes, in his deposition, that Kovac refers to "mitosis" (cell division) and the presence of two cells in a single well (*see* Ex. 2010, 219:3–220:7, 221:4–222:4), we find no reference in Kovac to levitation of cells that have been cultured within a microwell. Rather, Kovac refers to the asymmetrical shape of cells that had begun the process of mitosis at the time of levitation, and to the ability of removing two independent cells that had been trapped in the same chamber. Ex. 1007, 9326 ("Significantly aspheric cells, such as those undergoing mitosis, can be removed quickly, as their asymmetry makes them easier to remove via the fluid flow after they are levitated slightly."); *id.*, Fig. 3 (demonstrating that it is possible to "remove a single targeted cell residing in a doubly loaded well").

[22] Kovac addressed this problem by treating the microwells to prevent adhesion. *See* Ex. 2012 ¶¶ 53–54; Ex. 1007, 9323–24, 9326. But Petitioner's proposed combination does not include any surface treatment to Park's microwells. *See* Pet. 62–63; Ex. 2010, 150:18–151:2.

in the prior art that would have suggested this further modification to Kovac's method, and does not explain how a person of ordinary skill in the art would have known when to turn on the fluid flow. *See id.*[23]

Given the challenges that a person of ordinary skill in the art would have faced in adapting Kovac's cell levitation method for use in Park's very different microwells, and the lack of disclosure addressing these problems in either Kovac or Park, we find that Petitioner has not shown, by a preponderance of the evidence, that a person of ordinary skill in the art would have made the combination. Thus, we conclude that Petitioner has not shown that claim 1 is unpatentable as obvious over the combination of Kovac and Park.

For dependent claims 11, 16, 24, 26, 27, and 30, Petitioner relies on the teachings of Park and adds no further argument beyond the contentions in its obviousness ground based on Park alone (Ground 4). *See* Pet. 59–62. Thus, we find Petitioner's arguments as to these claims unpersuasive of obviousness and conclude that Petitioner has not shown these claims unpatentable. *See Fine*, 837 F.2d at 1076.

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[23] According to Petitioner's combination, the user would be observing the microwells "from above." *See* Pet. 63; Ex. 1002 ¶ 181. Under these circumstances, it is unclear how the user would have been able to determine whether the cell has reached a sufficient height not to be pulled back into the microwell after flow resumes.

IV. CONCLUSION

For the reasons above, Petitioner has not shown by a preponderance of the evidence that any challenged claim of the '408 patent is unpatentable under any ground of the Petition.

V. ORDER

In consideration of the foregoing, it is

ORDERED that claims 1, 6, 11, 16, 19, 24, 26, 27, and 30 of the '408 patent have not been shown to be unpatentable;

FURTHER ORDERED that parties to this proceeding seeking judicial review of our decision must comply with the notice and service requirements of 37 C.F.R. § 90.2.

In summary:

Claim(s)	35 U.S.C. §	Reference(s)/ Basis	Claims Shown Unpatentable	Claims Not Shown Unpatentable
1, 6, 11, 19, 24, 26, 27, 30	102(a), (e)	Dimov		1, 6, 11, 19, 24, 26, 27, 30
1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov		1, 6, 11, 19, 24, 26, 27, 30
1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov, Kovac		1, 6, 11, 19, 24, 26, 27, 30
1, 11, 16, 24, 26, 27, 30	103(a)	Park		1, 11, 16, 24, 26, 27, 30
1, 11, 16, 24, 26, 27, 30	103(a)	Park, Kovac		1, 11, 16, 24, 26, 27, 30
Overall Outcome				1, 6, 11, 16, 19, 24, 26, 27, 30

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Paper 41
Entered: July 20, 2023

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BERKELEY LIGHTS, INC.,
Petitioner,

v.

THE UNIVERSITY OF BRITISH COLUMBIA,
Patent Owner.

IPR2021-01249
Patent 10,087,408 B2

Before KRISTINA M. KALAN, CHRISTOPHER M. KAISER, and CHRISTOPHER L. OGDEN, *Administrative Patent Judges*.

OGDEN, *Administrative Patent Judge*.

JUDGMENT
Denying Petitioner's Request on Rehearing
35 U.S.C. § 314; 37 C.F.R. § 42.71

I. INTRODUCTION

Berkeley Lights, Inc. ("Petitioner") filed a Request for Rehearing of the Final Written Decision (Paper 38, "Dec." or "Decision") determining that Petitioner had not shown that any of claims 1, 6, 11, 16, 19, 24, 26, 27, and 30 of U.S. Patent No. 10,087,408 B2 (Ex. 1001, "the '408 patent") were unpatentable. Paper 40 ("Req. Reh'g").[1] Specifically, Petitioner requests rehearing on Grounds 2–5 asserting unpatentability under 35 U.S.C. § 103(a). *See id.* at 1. For the reasons that follow, the Request for Rehearing is *denied*.

II. STANDARD OF REVIEW

When a party requests rehearing on a final written decision, we "review the decision for an abuse of discretion." 37 C.F.R. § 42.71(d) (2022). There is an abuse of discretion if we have made "a clear error of judgment in weighing relevant factors or in basing [our] decision on an error of law or on clearly erroneous factual findings." *Bayer CropScience AG v. Dow AgroSciences LLC*, 851 F.3d 1302, 1306 (Fed. Cir. 2017) (quoting *Mentor Graphics Corp. v. Quickturn Design Sys., Inc.*, 150 F.3d 1374, 1377 (Fed. Cir. 1998)). In a request for rehearing, "[t]he burden of showing a

[1] We further refer to Patent Owner's Response (Paper 18, "PO Resp."), Petitioner's Reply to the Patent Owner Response (Paper 22, "Pet. Reply"), and Patent Owner's Sur-reply (Paper 29, "PO Sur-reply").

decision should be modified lies with the party challenging the decision," and "[t]he request must specifically identify all matters the party believes the Board misapprehended or overlooked, and the place where each matter was previously addressed" in the record. 37 C.F.R. § 42.71(d).

III. ANALYSIS

Petitioner's arguments in the Request for Rehearing concern limitation 1E, which recites "selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step." Dec. 7; Ex. 1001, 32:7–9; *see generally* Req. Reh'g. We address Petitioner's arguments below.

A. GROUND 2: CLAIMS 1, 6, 11, 19, 24, 26, 27, AND 30 AS OBVIOUS OVER DIMOV

1. Motivation to Modify Dimov to Selectively Recover Cells of Interest

As to the obviousness ground over Dimov, Petitioner asserts that we "misapprehended or overlooked Petitioner's arguments by demanding that Dimov *alone* provide" the proposed motivation to selectively recover cells of interest for further analysis outside the device. Req. Reh'g 2–3. (citing Dec. 25, 30–31). Rather, Petitioner asserts that this ground "is based on Dimov *plus the knowledge of a [person of ordinary skill in the art]*." *Id.* (citing Pet. Reply 8). Petitioner then reiterates its arguments relying the background knowledge in the art for the specific motivation to recover cells selectively. *Id.* at 3–4.

We disagree that we misapprehended Petitioner's reliance, in part, on the background knowledge in the art. We characterized Petitioner's

argument as "that claim 1 would have been obvious over Dimov, in view of the background knowledge of a person of ordinary skill in the art." Dec. 24 (citing Pet. 34–35; Ex. 1002 ¶ 106). We also stated that "[t]he main issue in dispute for this ground is whether there was a sufficient motivation *and sufficient background knowledge in the art*, at the time of the claimed invention, that a person of ordinary skill in the art would have modified Dimov's device to introduce selective recovery." Dec. 25 (emphasis added).

In the Decision, we did not find Petitioner's arguments persuasive in light of the background knowledge in the art. In particular, we found that Petitioner had not adequately shown that the background knowledge in the art would have been sufficient to inform a person of ordinary skill in the art how to apply the techniques such as "laser tweezers" that Dimov mentions briefly, in a way that would have allowed further analyses of cells outside Dimov's device. *See* Dec. 29–31. We also found that Petitioner had not persuasively shown that modifying Dimov's device to make its trenches accessible with a micropipette was within the background knowledge in the art at the time of the claimed invention. *See* Dec. 31–34.

Thus, we determine that Petitioner has not shown that we misapprehended or overlooked Petitioner's asserted motivation to modify Dimov in view of the background knowledge in the art.

2. Modifying Dimov to Recover a Cell from Dimov's "Common Output" or "Common Waste"

Petitioner argues that a person of ordinary skill in the art "would also be motivated to—and know how to—modify Dimov's device to recover a cell of interest, even if it were true that 'Dimov's device is a "lab on a chip" designed in general to retain cells while performing multiple experiments.'"

Req. Reh'g 4 (citing Dec. 29). Specifically, Petitioner argues that one of ordinary skill in the art, employing Dimov's listed techniques to remove a cell from a trench, "would know that the cell could be recovered from Dimov's 'common output' or 'common waste,' as they are convenient outlets for accessing cells." *Id.* at 4–5 (citing Pet. 35, 44; Pet. Reply 5). According to Petitioner, the Decision misapprehends "this evidence and overlooks a [person of ordinary skill in the art]'s ability to collect a *single* cell exiting from a device." *Id.* at 5 (citing Pet. 44; Ex. 1003, 3:2–26, Fig. 2; Ex. 1002 ¶ 128).

This argument is unpersuasive. In our Decision, we found that Petitioner had not shown that Dimov's teaching that the device can be modified to allow removal of particles from a trench through techniques such as "laser tweezers" was sufficient to teach selective recovery of cells suitable for use in further investigations such as sequencing or culturing. *See* Dec. 29–31; *see also* Dec. 24 (crediting Dr. Gale's testimony that there would have been substantial obstacles to recovering specific cells liberated from Dimov's trenches). Even if Dimov were read to include a teaching in that regard, we found that Petitioner's argument was missing any persuasive showing that the specifics of conducting such removal would have been within the ordinary level of skill and creativity in the art at the time of the claimed invention. *See* Dec. 29–31.

3. Recovering Cells via Micropipette Techniques

Petitioner next asserts that we failed to consider a modification of Dimov that involves "insert[ing] a micropipette into the trench and aspirat[ing] one or more cells . . . , for example, by piercing the top layer of PDMS (which is only 40 microns thick in Dimov's device) with a

micropipette." Req. Reh'g 5–6 (citing Ex. 1003, 5:37–39; Pet. 36–40; Pet. Reply 5, 8; Ex. 1038, 39:18–22). According to Petitioner, this is a different proposed modification than the one we considered in our Decision, which involved peeling back the top layer over a trench so that the trench could be accessed with the micropipette. *Id.* at 6 (citing Dec. 31–34).

Petitioner points to only one brief mention, in its Petition, of piercing the top layer of Dimov's device to retrieve cells with a micropipette. *Id.* at 6 (citing Pet. 40). That argument was not backed by any evidence other than a conclusory statement by Dr. Meinhart repeating the argument in the Petition. Pet. 40; Ex. 1002 ¶ 120. We do not credit that testimony because it lacks factual support. *Xerox Corp v. Bytemark, Inc.*, IPR2022-00624, Paper 9 (PTAB Aug. 24, 2022) (precedential) (the testimony of a declarant that merely repeats, verbatim, conclusory assertions from a petition, without citing additional supporting evidence or providing technical reasoning to support the testimony, is entitled to little weight). In particular, to the extent that Petitioner argues on rehearing that Dimov's top layer could have been pierced successfully by a micropipette because it was only 40 microns thick, Petitioner did not properly make this argument during trial or provide adequate evidentiary support.

Petitioner's related argument is that it would have been obvious to make the top layer of Dimov's device peelable. Req. Reh'g 6. This argument relies on Han as evidence of the purported state of knowledge in the art soon after the time of the claimed invention. *Id.*; Dec. 31–34. On rehearing, Petitioner argues that "[i]t was legal error for the Decision to discredit this probative evidence of a [person of ordinary skill in the art]'s motivation to selectively aspirate cells from a device by micropipette." Req. Reh'g 6.

We did not disregard Han as potentially probative evidence. Rather, after noting that Han was not prior art, we found that "Petitioner has not shown that the peelable layer in Han's device reflects the background knowledge of a person of ordinary skill in the art at the time of the claimed invention." *Id.* at 33 (citing PO Sur-reply 7–8). Specifically, we agreed with Patent Owner that Han characterized its microfluidic device as "novel" and distinguished it from previous devices known in the art. *Id.* (citing PO Sur-reply 7–8; Ex. 1009, 2853).

For these reasons, we are not persuaded of any abuse of discretion in our determination that Petitioner failed to show that any of claims 1, 6, 11, 19, 24, 26, 27, and 30 would have been obvious over Dimov in view of the knowledge of one of ordinary skill in the art.

B. GROUND 3: CLAIMS 1, 6, 11, 19, 24, 26, 27, AND 30 AS OBVIOUS OVER DIMOV AND KOVAC

Petitioner asserts that we misapprehended its argument for combining Dimov and Kovac. According to Petitioner, our decision only considered "select[ing] Dimov's microfluidic device for use in Kovac's cell levitation method," whereas Petitioner's true argument was that a person of ordinary skill in the art would have modified Dimov's device to incorporate Kovac's laser levitation teachings. Req. Reh'g 9–10 (quoting Dec. 49); *see also id.* at 7–9 (reiterating Petitioner's argument in the Petition and Reply).

We disagree that we misapprehended Petitioner's argument by reversing the order of the references. Although we agreed with Patent Owner that Petitioner had failed to show that Dimov's microfluidic device was a suitable starting point for use with Kovac's cell levitation method (*see* Dec. 49), we summarized Petitioner's position as follows: "Petitioner asserts

that the motivation to modify Dimov's microfluidic device in view of Kovac's levitation method would have been to allow a user to selectively recover cells of interest for later analysis outside the device." Dec. 48 (citing Pet. 43); *see* Req. Reh'g 8–10. We also held that Petitioner had not shown evidence in Dimov "that a person of ordinary skill in the art would have selected Kovac's *specific* laser levitation method for use in Dimov's device to recover cells of interest for further analysis." Dec. 49. We further found that Kovac's laser levitation method was highly dependent on a number of parameters, and Petitioner had not persuasively shown that a person of ordinary skill in the art would have had the specific motivation or ability to discover those parameters to levitate cells in Dimov's device in a way that avoided unacceptable cell damage and thus destroyed the very motivation that Petitioner asserts for using Kovac's levitation method. *See* Dec. 49–51. We also found, as with the obviousness ground based on Dimov alone, that Petitioner had not shown it would have been feasible to successfully recover any liberated cells from Dimov's device. Dec. 52.

Next, Petitioner argues that, by focusing on the difficulties a person of ordinary skill in the art would have faced in combining Dimov with Kovac, our analysis confuses "motivation to combine" with "reasonable expectation of success." Req. Reh'g 11–12. We disagree. As Petitioner acknowledges, our Decision did not rely on whether Petitioner had shown a reasonable expectation of success. *See id.* at 7 (citing Dec. 51 n.15). In determining whether there would have been a motivation to combine Dimov with Kovac, we must consider "the background knowledge possessed by a person having ordinary skill in the art" and "the inferences and creative steps that a person of ordinary skill in the art would employ." *KSR Int'l Co. v. Teleflex Inc.*, 550

U.S. 398, 418 (2007). In light of these considerations, we found that Petitioner failed to show that a person of ordinary skill in the art (as Petitioner articulated it) would have had the background necessary to adapt Kovac's laser parameters for use in Dimov's device to successfully recover usable cells from Dimov's output or waste streams. *See* Dec. 50–52.

For these reasons, we are not persuaded of any abuse of discretion in our determination that Petitioner failed to show that any of claims 1, 6, 11, 19, 24, 26, 27, and 30 would have been obvious over Dimov in view of Kovac.

C. GROUND 4: CLAIMS 1, 11, 16, 24, 26, 27, AND 30 AS OBVIOUS OVER PARK

For the obviousness ground based on Park, Petitioner argues that our statement that "Petitioner has not pointed to anything in Park that teaches selectively recovering cells" is in error because "[t]hat may be relevant to anticipation, but not obviousness." Req. Reh'g 12 (quoting Dec. 58). We disagree. That Petitioner has not identified any teaching about selective recovery in Park, itself, means that Petitioner must rely on the background knowledge in the art to establish limitation 1E.

In that regard, Petitioner reiterates its arguments that we found unpersuasive, without identifying any error of law or clear factual error in our Decision. *See* Req. Reh'g 12–13 (citing Pet. 57–59). Thus, we are not persuaded of abuse of discretion in our determination that Petitioner failed to show that claims 1, 11, 16, 24, 26, 27, or 30 would have been obvious over Park.

D. GROUND 5: CLAIMS 1, 11, 16, 24, 26, 27, AND 30 AS OBVIOUS OVER PARK AND KOVAC

For the ground based on the combination of Park and Kovac, Petitioner contends that "the Decision's analysis does not separately address either motivation to combine them or the reasonable expectation of success of doing so; indeed, it does not even mention either." Req. Reh'g 13–14 (citing Dec. 68–70).

We disagree that we failed to address whether there was a motivation to combine Park and Kovac the way that Petitioner proposes. For example, as with Ground 3, we found that Petitioner had not persuasively shown that a person of ordinary skill in the art would have had the background necessary to determine suitable laser parameters to achieve Petitioner's proposed combination. *See* Dec. 68. We also found that Petitioner failed to persuasively explain how a person of ordinary skill in the art would have been motivated to address the problems identified by Patent Owner's expert Dr. Gale, which would have worked against the ability to levitate and recover individual cells from Park's device. Dec. 68–70.

Petitioner also argues that, by "merely cit[ing] to [our] earlier discussion of laser parameters in Ground 3 (Decision at 68), [we] overlooked two important facts" suggesting "that Kovac's techniques could recover a *single* cell from Park's microwell": (1) that "Kovac demonstrated that it is possible to 'remove a single targeted cell residing in a doubly loaded well,'" and (2) that "Park's microwells have a 'depth of 20 μm,' whereas Kovac's microwells are '35 μm in depth.'" Req. Reh'g 14 (quoting Ex. 1006, 266; and then quoting Ex. 1007, 9326) (citing Ex. 1007, 9326, Fig. 3; Pet. 63). According to Petitioner, "[t]he Decision errs in concluding

that it would require 'additional inventive steps' to 'adapt[] Kovac's levitation method for use in Park's device . . . .'" *Id.* (citing Dec. 68).

We disagree. The question is not whether Kovac's techniques "could" recover a single cell from Park's microwell, but whether a person of ordinary skill in the art would have been motivated to make that combination in a predictable way, in light of their background knowledge. *See KSR*, 550 U.S. at 417 ("The mere fact that references can be combined or modified does not render the resultant combination obvious unless the results would have been predictable to one of ordinary skill in the art."). Although Kovac discusses the removal of single cells from Kovac's microwells, our Decision found that Petitioner failed to show that a person of skill in the art would have been able to apply Kovac's teachings predictably within Park's substantially different device. *See* Dec. 68–70 & nn.21–23.

In particular, Petitioner argues that "the Decision overlooks the fact that the lateral gradient force of Kovac's laser column counteracts lateral flows in Park's device." Req. Reh'g. 15 (citing Pet. Reply 24; Ex. 1039 ¶¶ 191, 194; Ex. 1007, 9325). Similarly, Petitioner contends that "the Decision overlooked Kovac's teaching that wall effects can be overcome by slightly increasing the power of the laser, so any resistance to vertical movement would not be problematic to a" person of ordinary skill in the art. *Id.* (citing Pet. Reply 24; Ex. 1039 ¶ 195). These arguments concern Petitioner's response to Patent Owner's argument (supported by testimony by Dr. Gale) that lateral flow and wall effects would hamper the ability to levitate cells in Park's microwells. Pet. Reply 24 (citing PO Resp. 49; Ex. 2012 ¶ 161); PO Resp. 46–50 & nn.18–19 (citing Ex. 2012 ¶¶ 161–166).

Petitioner had argued that "Patent Owner fails to explain how resisting lateral movement would affect selective recovery by levitation, and does not establish that any resistance to vertical movement would be problematic either." Pet. Reply 24 (citing Ex. 1039 ¶¶ 190–196). Petitioner's support for this rebuttal argument was five pages of expert declaration testimony that Petitioner did not explain and did not otherwise discuss. *See* 37 CFR 42.6(a)(3) ("Arguments must not be incorporated by reference from one document into another document."). We stated in our Decision that this argument "improperly shifts the burden of persuasion to Patent Owner." Dec. 65 n.19. In any event, we did not find Petitioner's argument or Dr. Meinhart's testimony persuasive, and instead, "we credit[ed] Dr. Gale's testimony that [lateral flow effects] would have worked against any laser levitation." Dec. 69 (citing Ex. 2012 ¶¶ 163–164; Ex. 1006, 266, Fig. 2a). We also "credit[ed] Dr. Gale's testimony that wall effects, or adhesion between cells and walls or other cells, would likely occur after culturing the cells in Park's microwells, and would have been complicating factors for which neither Park nor Kovac provide solutions." *Id.* (footnote omitted).

Petitioner also argues on rehearing that, if the asserted lateral gradient force of Kovac's laser were insufficient to counteract lateral flow forces, "an increased laser spot size the size of Park's microwell would be [a sufficient solution], which requires only a simple change of lens, not any substantial experimentation, which the Decision (at 68) overlooks as well." Req. Reh'g 15 (citing Pet. Reply 24; Ex. 1039 ¶¶ 107–108, 192).

Although Dr. Meinhart briefly mentions this in his declaration (*see* Ex. 1039 ¶ 192), Petitioner has not shown that it made this argument during trial, except to the extent that Petitioner improperly incorporated it by

reference from Dr. Meinhart's declaration. *See* Reply 24 (citing Ex. 1039 ¶¶ 190–196); 37 CFR § 42.6(a)(3).

In any event, we do not credit Dr. Meinhart's testimony on this point because he provides no factual support. *See* Ex. 1039 ¶ 192; 37 C.F.R. § 42.65(a) ("Expert testimony that does not disclose the underlying facts or data on which the opinion is based is entitled to little or no weight."). As Patent Owner pointed out in its Sur-Reply, Dr. Meinhart acknowledged at deposition that increasing the spot size would have reduced the lateral gradient force of the levitation laser. PO Sur-reply 23 (citing Ex. 2013, 344:18–345:5). We agree with Patent Owner that "Dr. Meinhart . . . did not consider how this reduction in force would have affected the laser's ability to levitate a cell in Park's well successfully (especially with cultured cells through Park's recirculation pattern)." *Id.*

Finally, Petitioner contends that "[t]he Decision recognizes that Kovac's laser is applied for up to about 30 seconds . . . but overlooks . . . the fact that a [person of ordinary skill in the art] would simply turn on the flow in Park's device after this amount of time, because a [person of ordinary skill] would know from Kovac that this was sufficient time to levitate a cell out of Park's shallower, 20-μm-deep microwells." Req. Reh'g 14 (citing Dec. 37, 70 n.23). According to Petitioner, a person of ordinary skill in the art "is not an uncreative automaton." *Id.* at 14–15 (citing *KSR*, 550 U.S. at 421).

As we stated in our Decision, Petitioner's theory that a person of ordinary skill would have begun Kovac's levitation procedure with fluid flow turned off, and then turned the fluid on during the levitation, was a new argument first made in the Reply. *See* Dec. 69. Nevertheless, we also found

that theory unpersuasive because "Petitioner does not point to anything in the prior art that would have suggested this further modification to Kovac's method, and does not explain how a person of ordinary skill in the art would have known when to turn on the fluid flow," especially considering that a user would have been observing the microwells from above. Dec. 69–70 & n.23.

On rehearing, Petitioner proposes for the first time that a person of ordinary skill in the art would have had reason to select "about 30 seconds" as the duration to perform levitation before turning the flow back on in Park's device. Req. Reh'g 14. Petitioner does not support that argument with any citations other than to our Decision, and in any event, it is untimely.

For these reasons, we are not persuaded of abuse of discretion in our determination that Petitioner failed to show that any of claims 1, 11, 16, 24, 26, 27, and 30 would have been obvious over Park in view of Kovac.

IV. CONCLUSION

For the above reasons, Petitioner has not shown abuse of discretion in our determination that Petitioner had not shown that any of claims 1, 6, 11, 16, 19, 24, 26, 27, and 30 of the '408 patent are unpatentable under the grounds of the Petition.

V. ORDER

In consideration of the foregoing, it is hereby:

ORDERED that Petitioner's Request for Rehearing is *denied*.

Outcome of Decision on Rehearing:

Claim(s)	35 U.S.C. §	Reference(s)/ Basis	Denied	Granted
1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov	1, 6, 11, 19, 24, 26, 27, 30	
1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov, Kovac	1, 6, 11, 19, 24, 26, 27, 30	
1, 11, 16, 24, 26, 27, 30	103(a)	Park	1, 11, 16, 24, 26, 27, 30	
1, 11, 16, 24, 26, 27, 30	103(a)	Park, Kovac	1, 11, 16, 24, 26, 27, 30	
Overall Outcome			1, 6, 11, 16, 19, 24, 26, 27, 30	

Final Outcome of Final Written Decision after Rehearing:

Claim(s)	35 U.S.C. §	Reference(s)/ Basis	Claims Shown Unpatentable	Claims Not Shown Unpatentable
1, 6, 11, 19, 24, 26, 27, 30	102(a), (e)	Dimov		1, 6, 11, 19, 24, 26, 27, 30
1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov		1, 6, 11, 19, 24, 26, 27, 30
1, 6, 11, 19, 24, 26, 27, 30	103(a)	Dimov, Kovac		1, 6, 11, 19, 24, 26, 27, 30
1, 11, 16, 24, 26, 27, 30	103(a)	Park		1, 11, 16, 24, 26, 27, 30
1, 11, 16, 24, 26, 27, 30	103(a)	Park, Kovac		1, 11, 16, 24, 26, 27, 30
Overall Outcome				1, 6, 11, 16, 19, 24, 26, 27, 30

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US010087408B2

(12) **United States Patent**
Hansen et al.

(10) **Patent No.: US 10,087,408 B2**
(45) **Date of Patent: Oct. 2, 2018**

(54) **SYSTEM AND METHOD FOR MICROFLUIDIC CELL CULTURE**

(75) Inventors: **Carl L. G. Hansen**, Vancouver (CA); **Veronique Lecault**, Vancouver (CA); **James M. Piret**, Vancouver (CA); **Anupam Singhal**, Mississauga (CA)

(73) Assignee: **The University of British Columbia**, Vancouver (CA)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 1032 days.

(21) Appl. No.: **13/178,395**

(22) Filed: **Jul. 7, 2011**

(65) **Prior Publication Data**

US 2012/0009671 A1 Jan. 12, 2012

Related U.S. Application Data

(60) Provisional application No. 61/362,213, filed on Jul. 7, 2010.

(51) **Int. Cl.**
C12N 5/0789 (2010.01)
C12M 3/00 (2006.01)
C12M 3/06 (2006.01)

(52) **U.S. Cl.**
CPC ***C12M 23/16*** (2013.01)

(58) **Field of Classification Search**
CPC C12M 23/16; Y10T 436/25; B01L 2200/0647; B01L 2200/0668; B01L 2300/0816; B01L 2300/0851; B01L 2300/0887; B01L 2400/0457; B01L 2400/0472; B01L 2200/10
See application file for complete search history.

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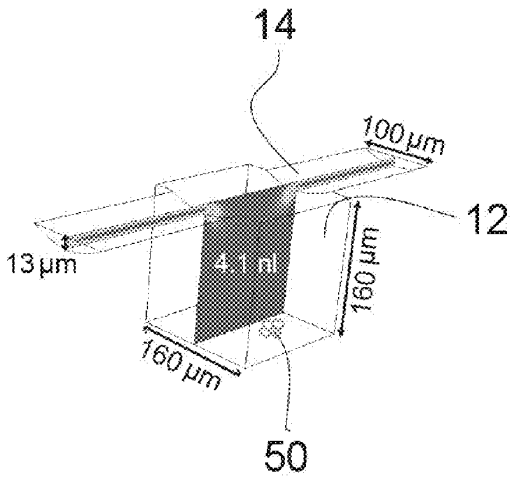
(57) **ABSTRACT**

Microfluidic devices and methods for perfusing a cell with perfusion fluid are provided herein, wherein the gravitational forces acting on the cell to keep the cell at or near a retainer or a retaining position exceed the hydrodynamic forces acting on the cell to move it toward an outlet.

34 Claims, 21 Drawing Sheets



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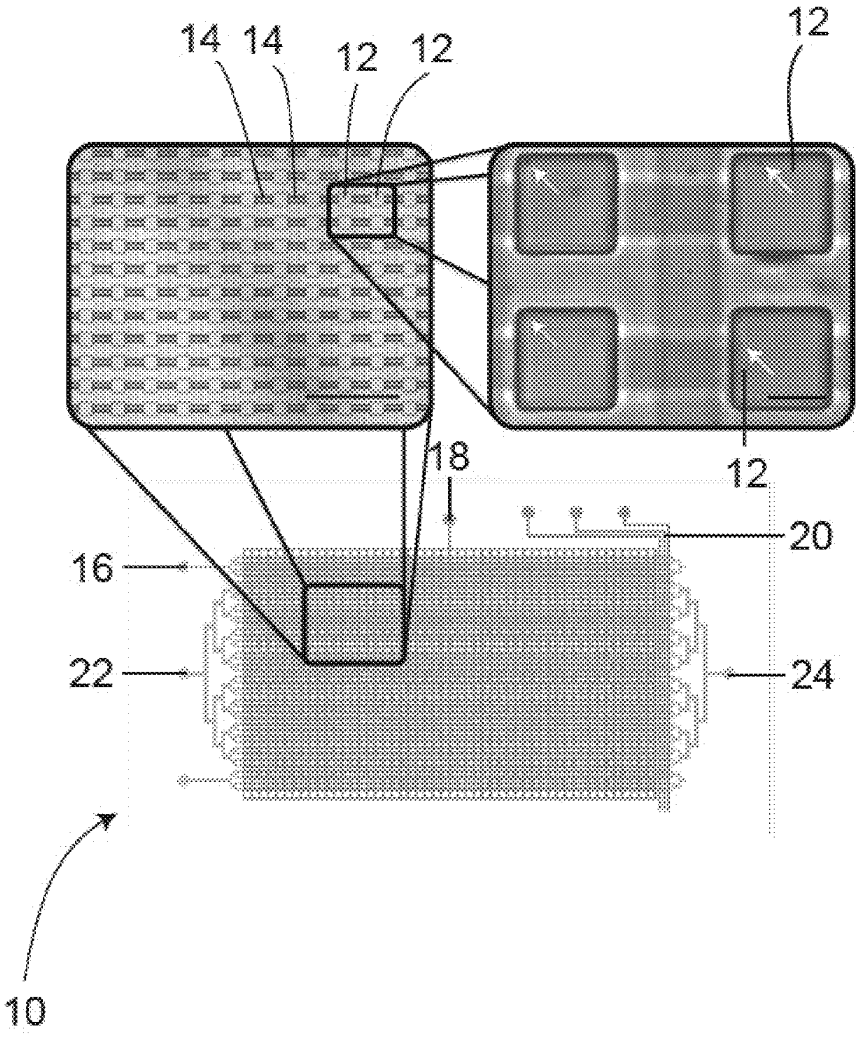


FIGURE 1

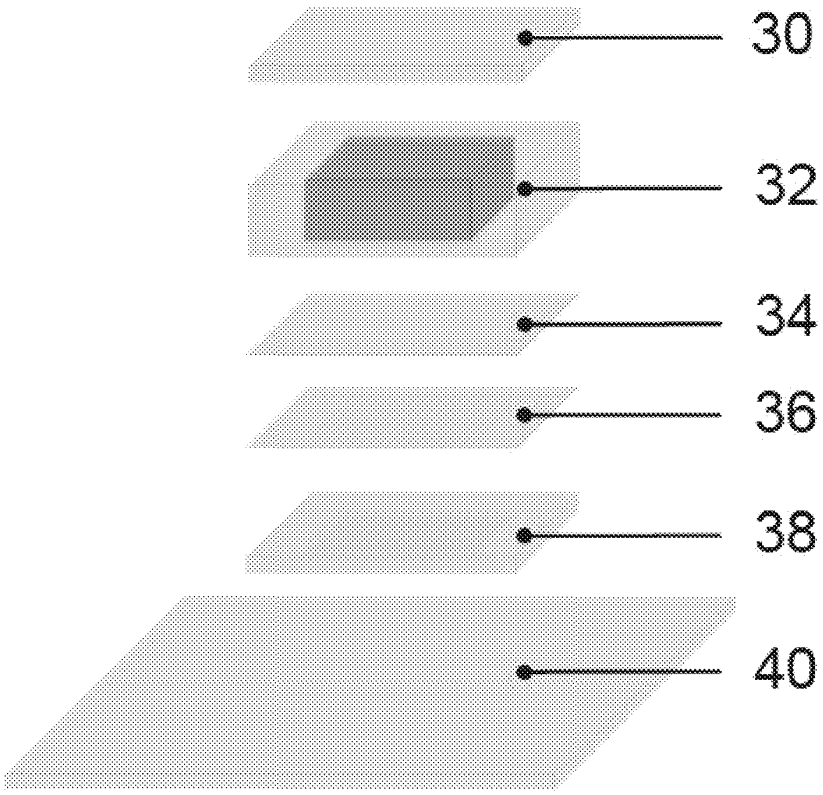
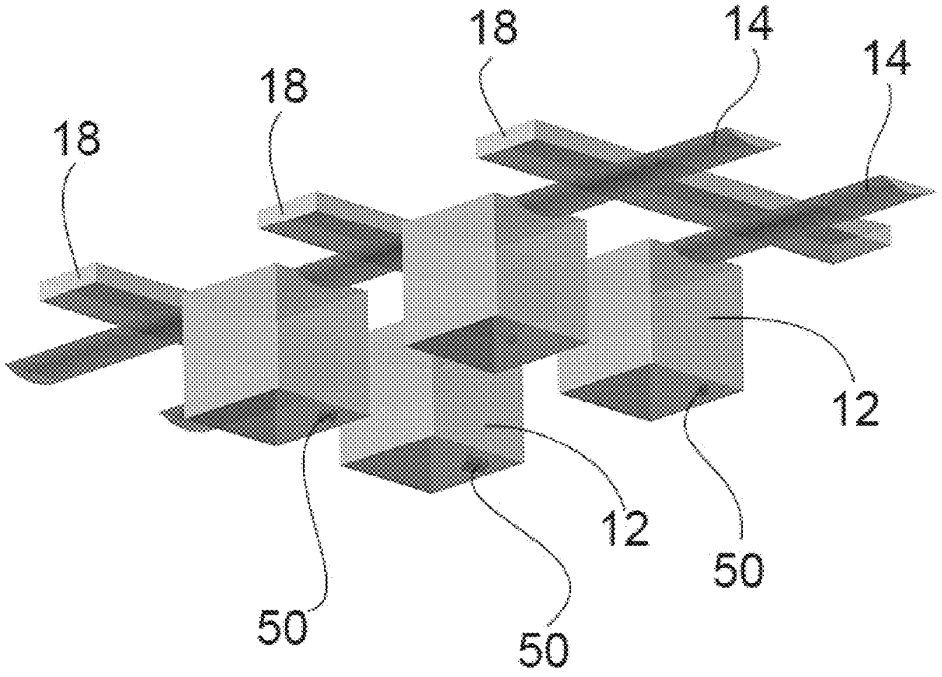


FIGURE 2



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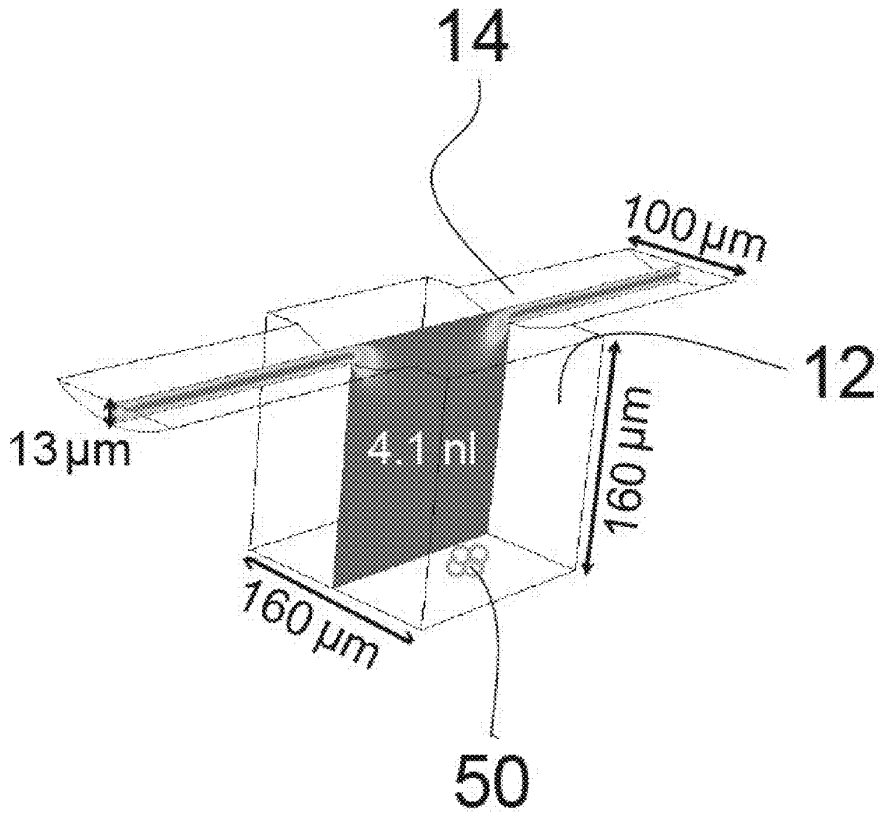


FIGURE 4

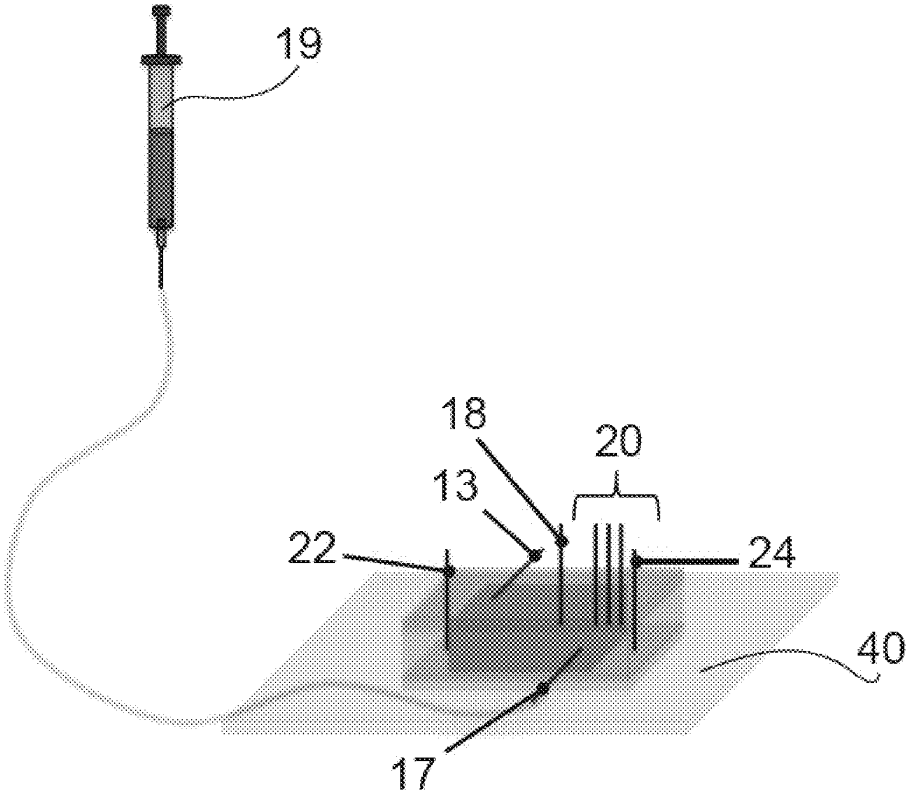


FIGURE 5

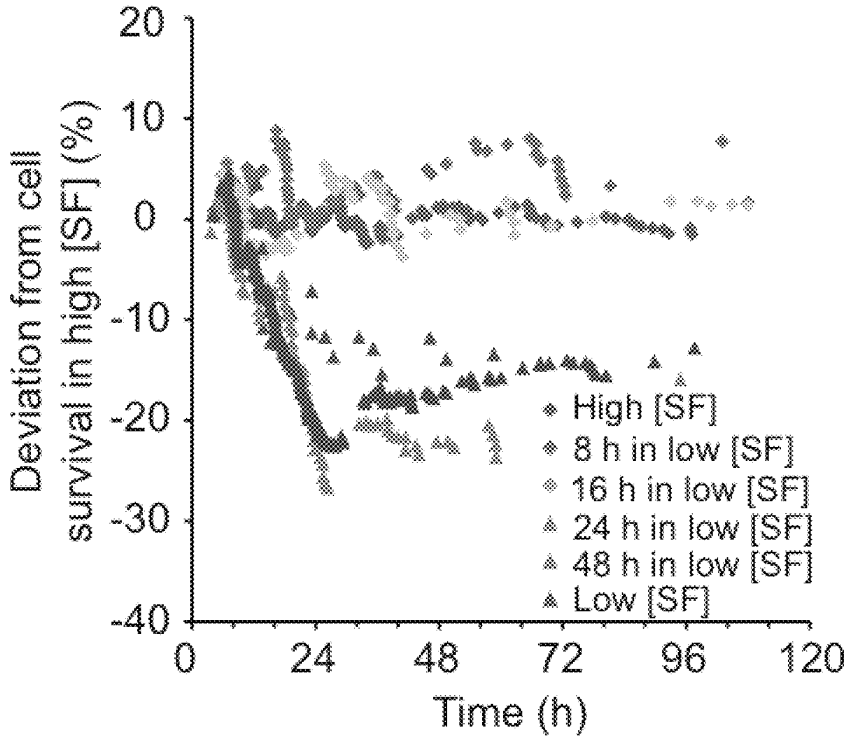

Deviation from cell survival in high [SF] (%)
20
10
0
-10
-20
-30
-40
0
24
48
72
96
120
Time (h)
High [SF]
8 h in low [SF]
16 h in low [SF]
24 h in low [SF]
48 h in low [SF]
Low [SF]

FIGURE 6

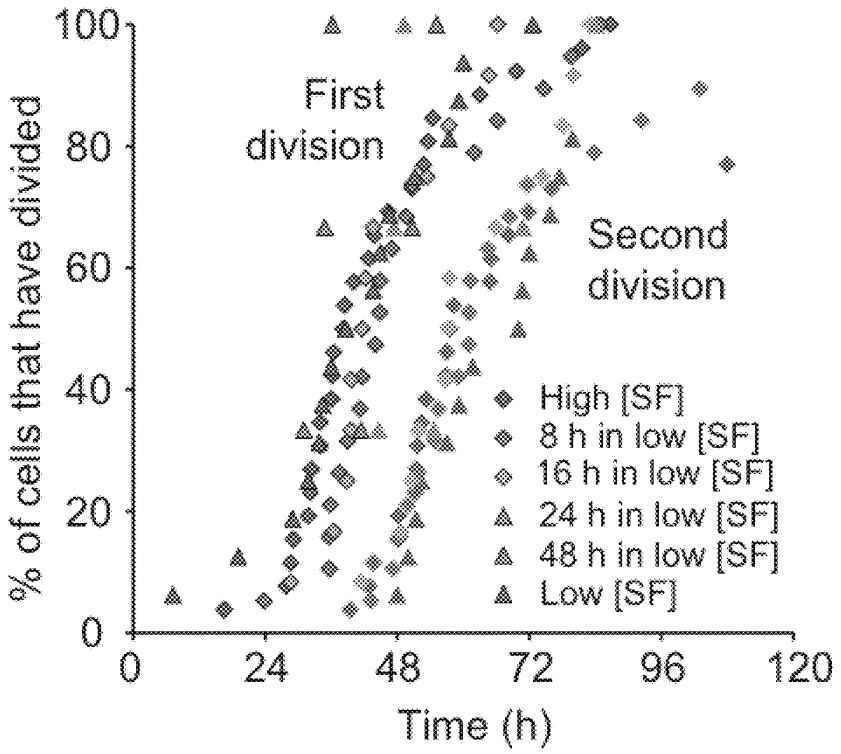

100
80
60
40
20
0
% of cells that have divided
First division
Second division
High [SF]
8 h in low [SF]
16 h in low [SF]
24 h in low [SF]
48 h in low [SF]
Low [SF]
0
24
48
72
96
120
Time (h)

FIGURE 7

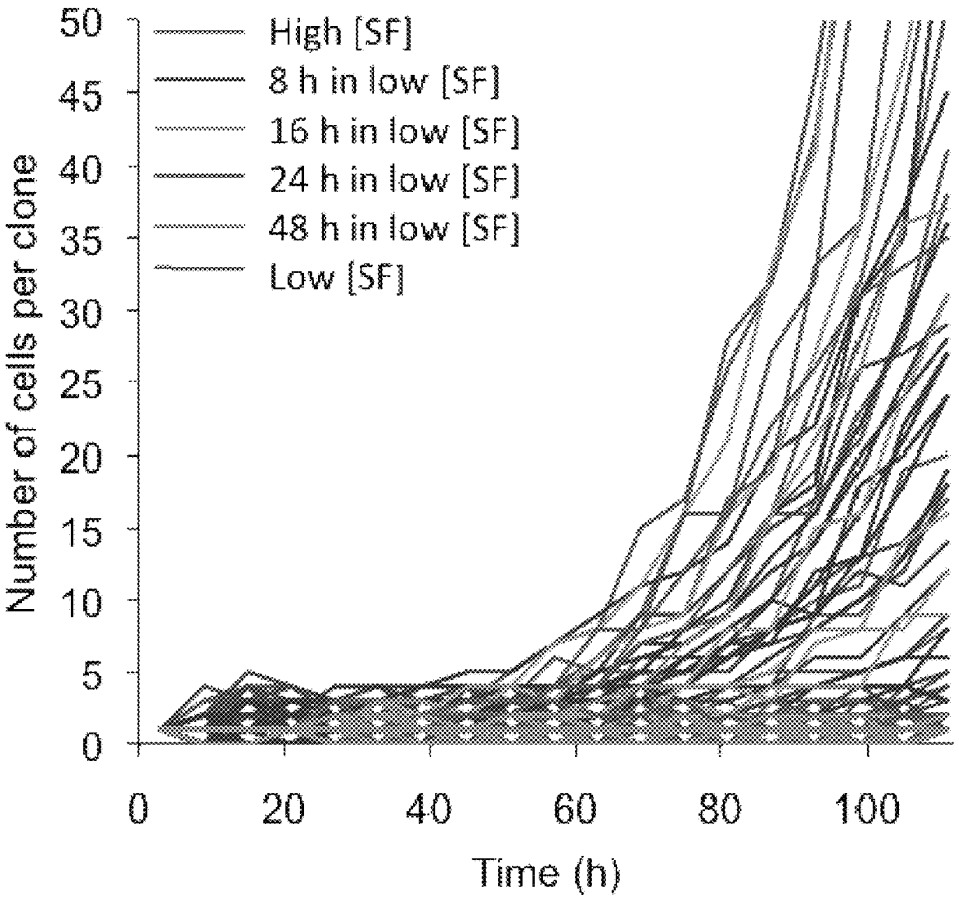
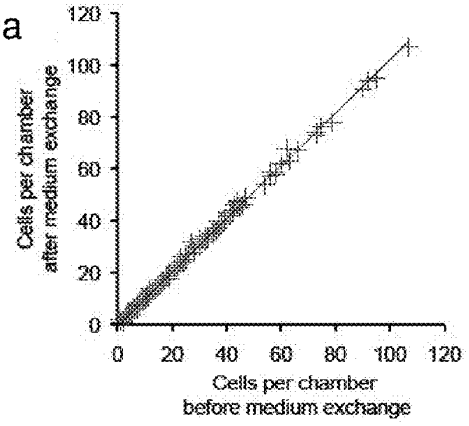
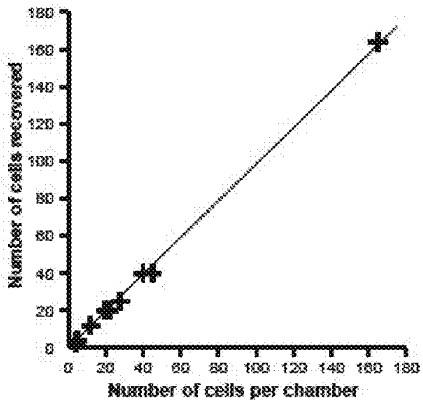

High [SF]
8 h in low [SF]
16 h in low [SF]
24 h in low [SF]
48 h in low [SF]
Low [SF]
Number of cells per clone
0
5
10
15
20
25
30
35
40
45
50
0
20
40
60
80
100
Time (h)

FIGURE 8

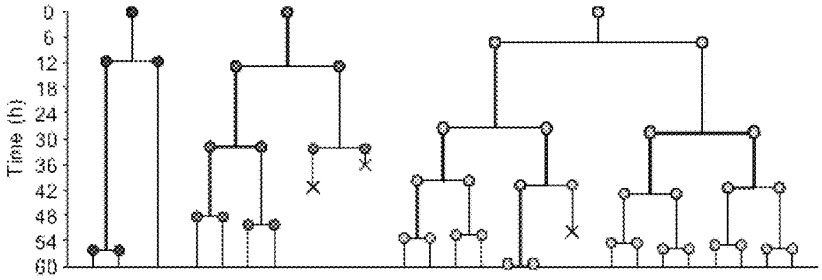
9a



9b



9c



FIGURES 9a-9c

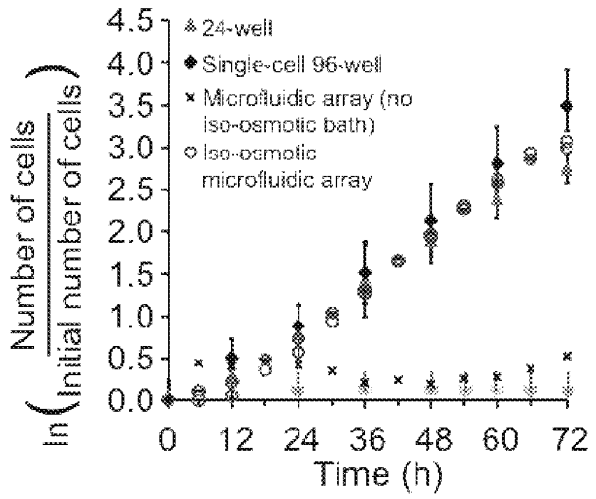

4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
12
24
36
48
60
72
Time (h)
ln(Number of cells / Initial number of cells)
24-well
Single-cell 96-well
Microfluidic array (no iso-osmotic bath)
Iso-osmotic microfluidic array

FIGURE 10

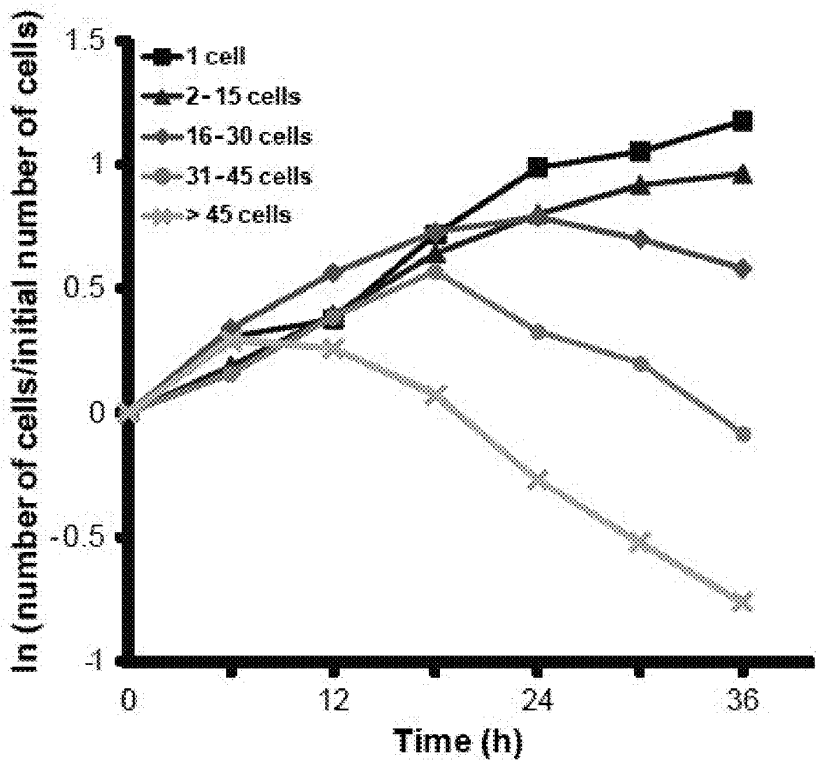
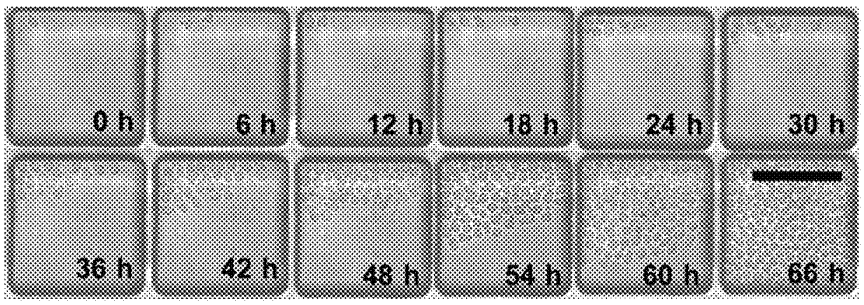
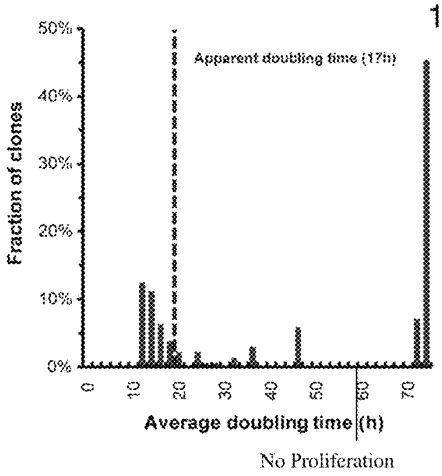

ln (number of cells/initial number of cells)
1.5
1
0.5
0
-0.5
-1
1 cell
2-15 cells
16-30 cells
31-45 cells
> 45 cells
0
12
24
36
Time (h)

FIGURE 11

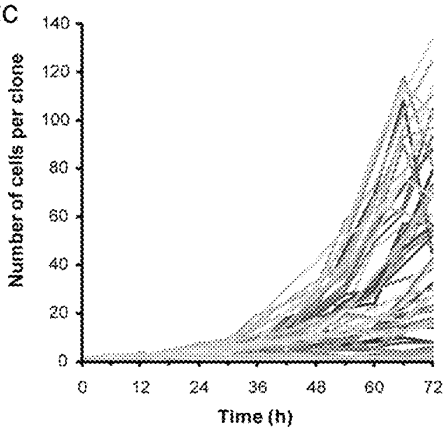
12a



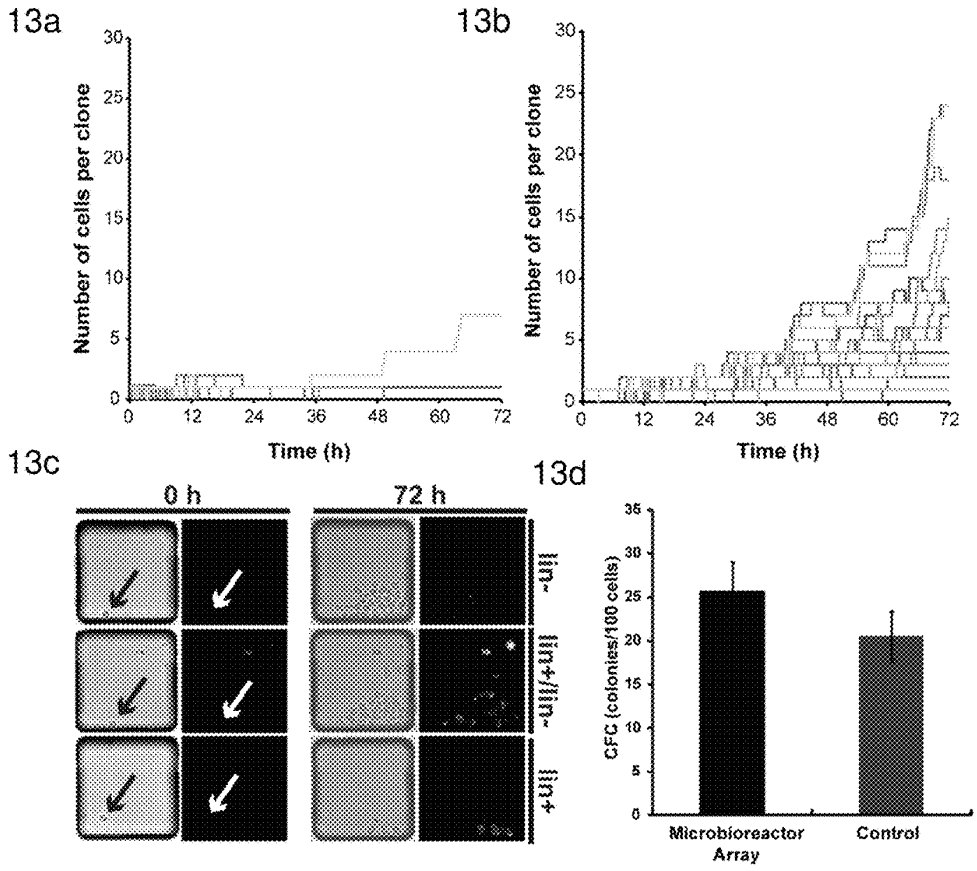
12b



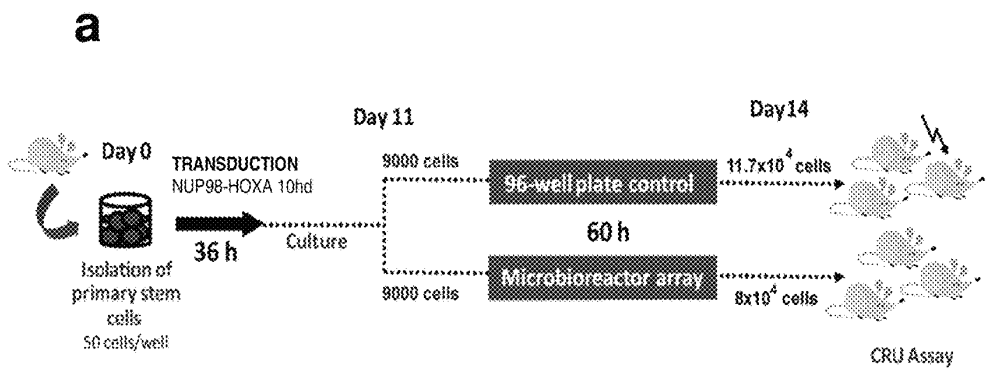
12c



FIGURES 12a-c



FIGURES 13a-d


a
Day 0
TRANSDUCTION
NUP98-HOXA 10hd
36 h
Culture
Isolation of primary stem cells
50 cells/well
Day 11
9000 cells
96-well plate control
9000 cells
Microbioreactor array
60 h
Day14
11.7x10^4 cells
8x10^4 cells
CRU Assay

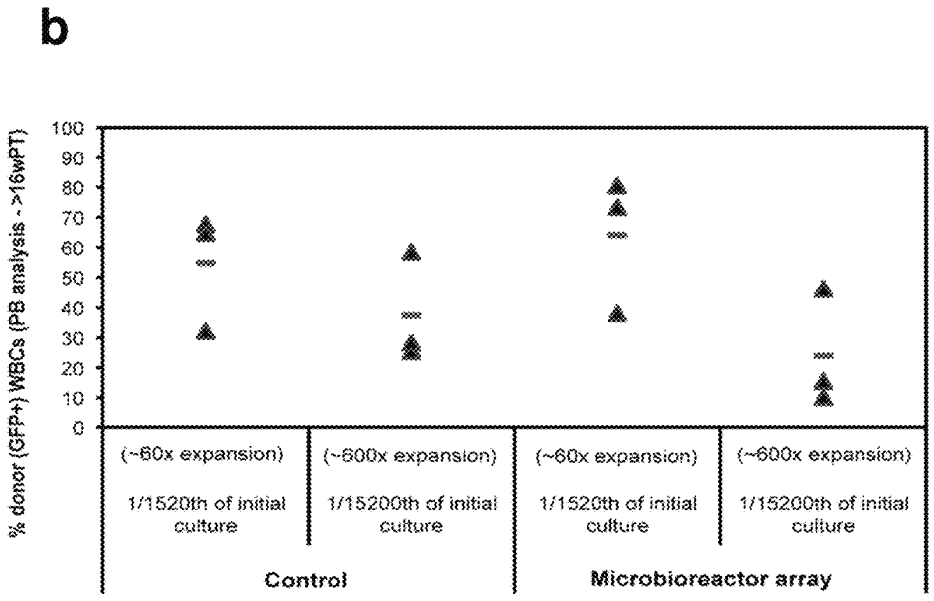
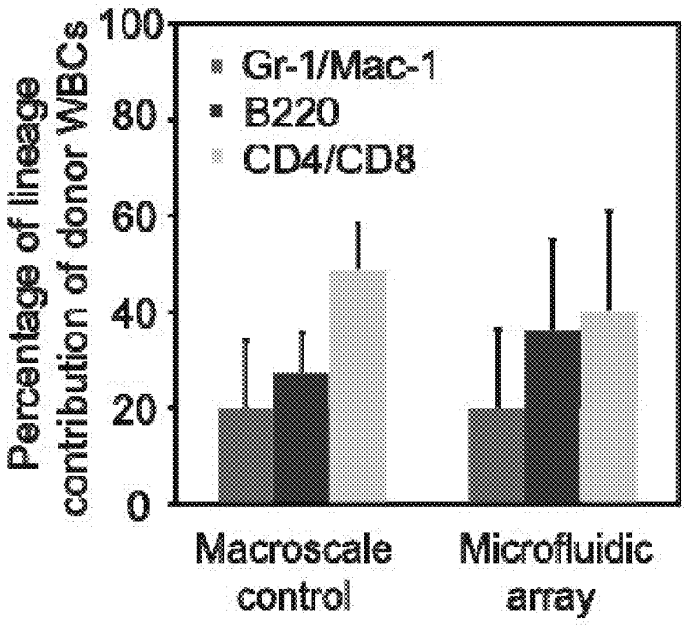
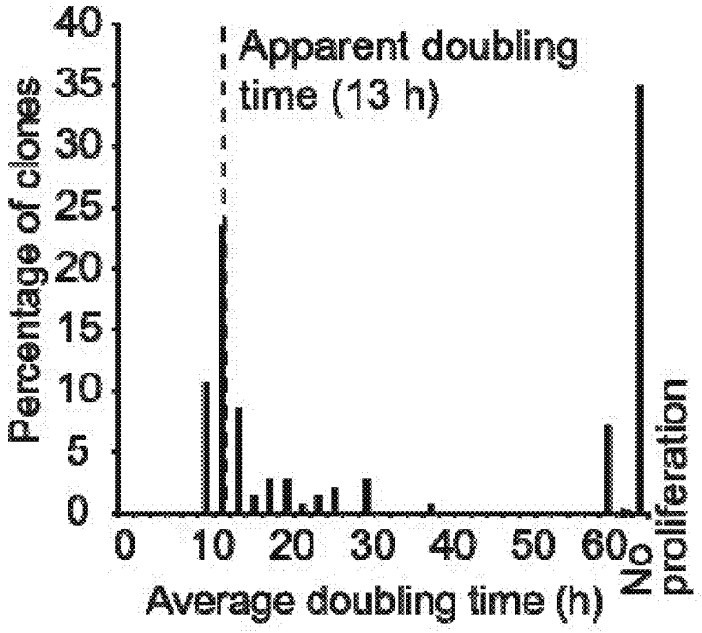

b
% donor (GFP+) WBCs (PB analysis - >16wPT)
100
90
80
70
60
50
40
30
20
10
0
(~60x expansion)
1/1520th of initial culture
(~600x expansion)
1/15200th of initial culture
(~60x expansion)
1/1520th of initial culture
(~600x expansion)
1/15200th of initial culture
Control
Microbioreactor array

FIGURE 14

15a



15b



FIGURES 15a-b

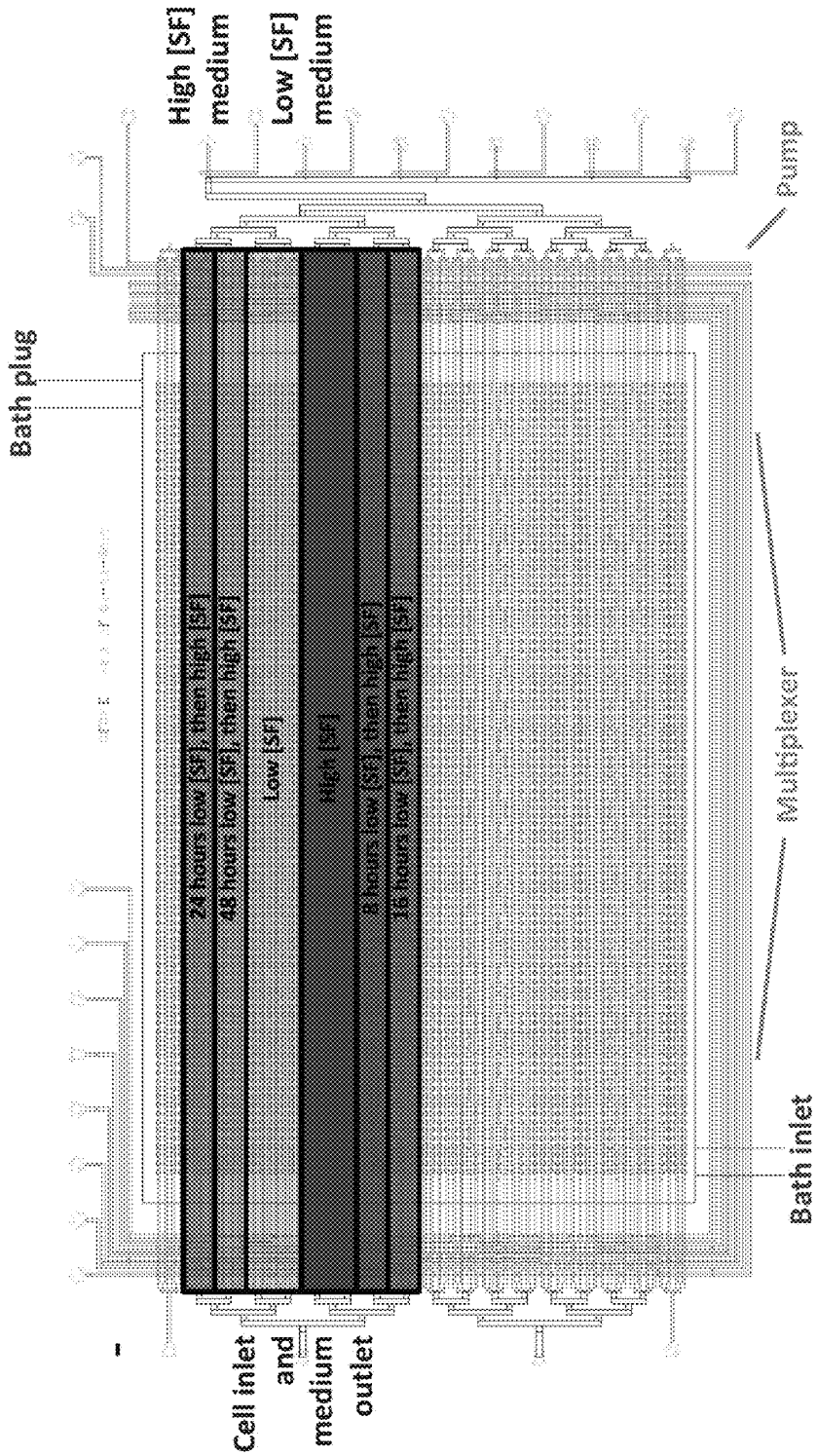
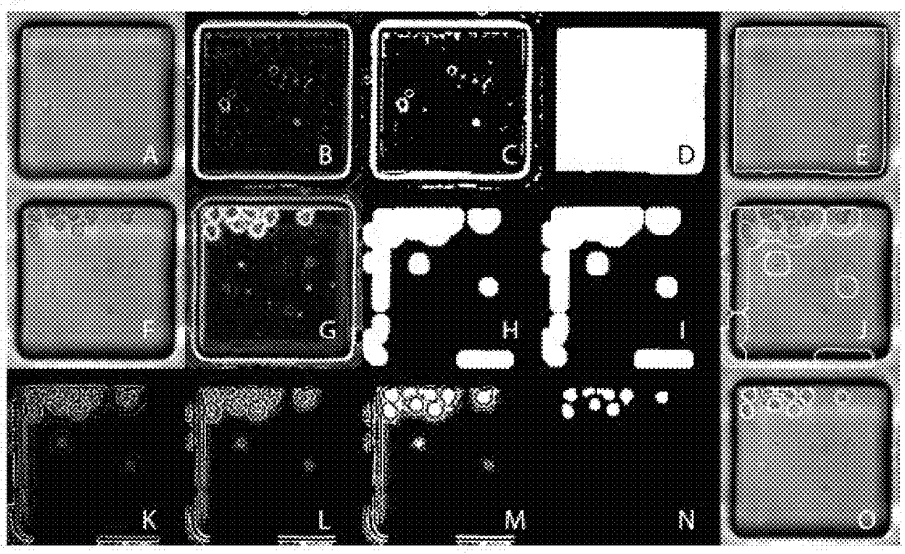
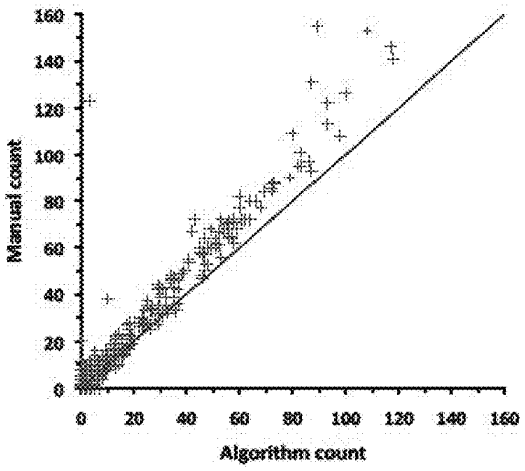

High [SF] medium
Low [SF] medium
Pump
Bath plug
24 hours low [SF], then high [SF]
48 hours low [SF], then high [SF]
Low [SF]
High [SF]
8 hours low [SF], then high [SF]
16 hours low [SF], then high [SF]
Multiplexer
Bath inlet
Cell inlet and medium outlet

FIGURE 16

17a



17b



FIGURES 17a-b

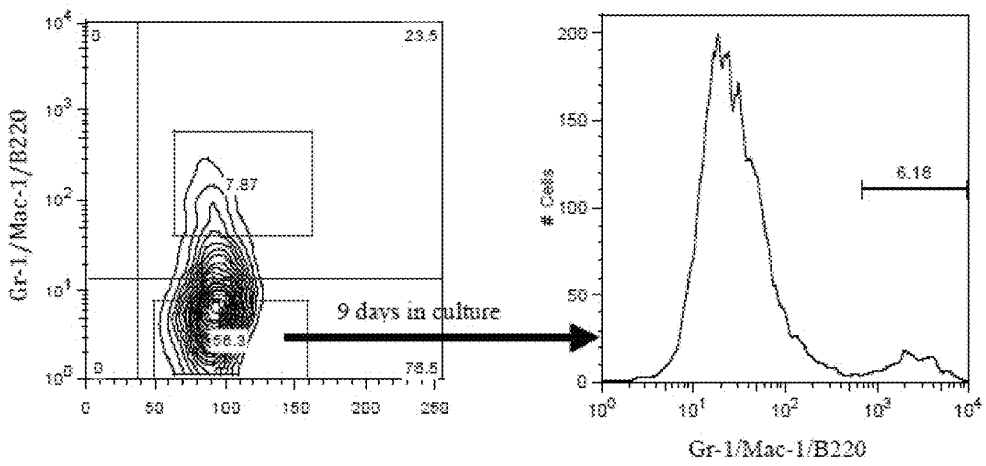

Gr-1/Mac-1/B220
23.6
7.87
56.3
78.5
9 days in culture
Cells
6.18
Gr-1/Mac-1/B220

FIGURE 18

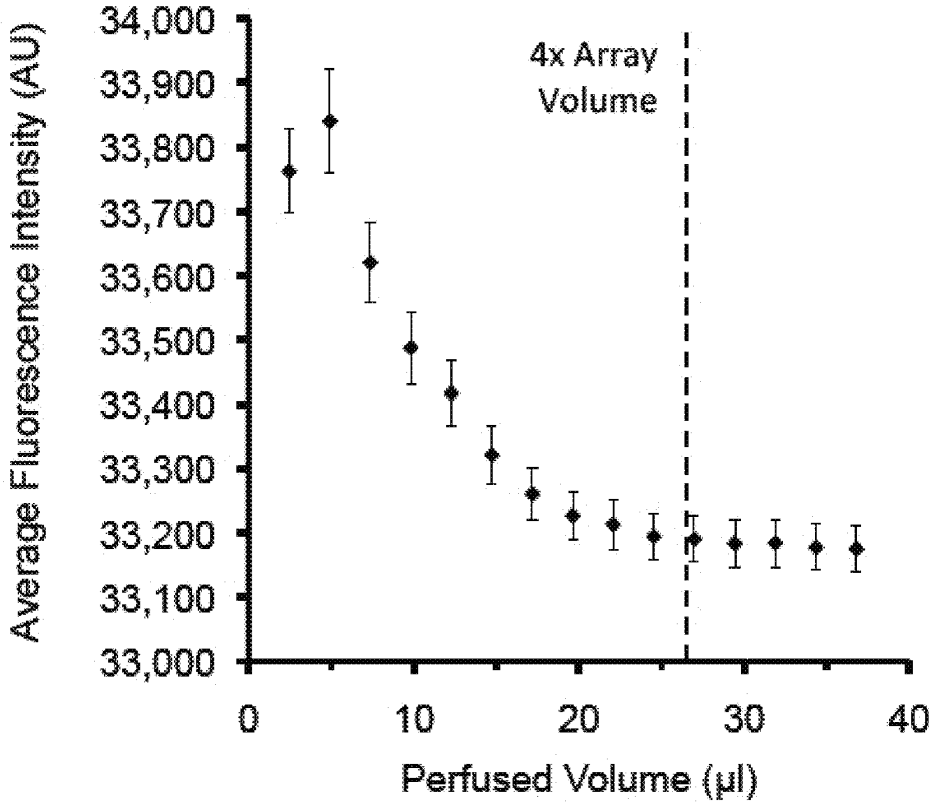
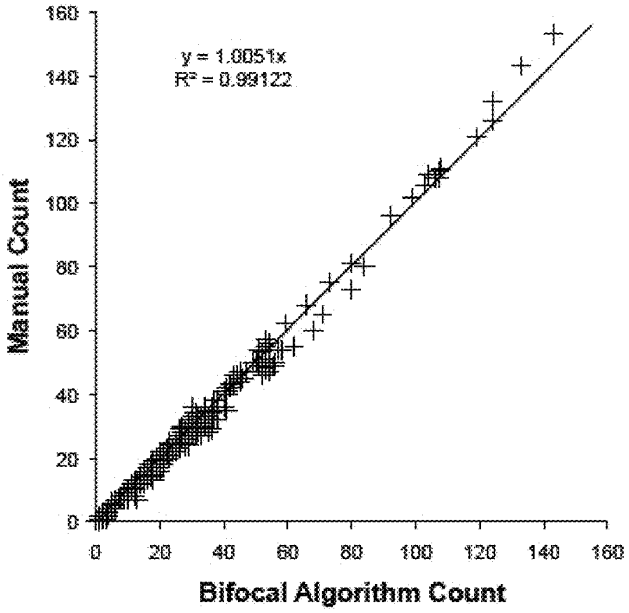
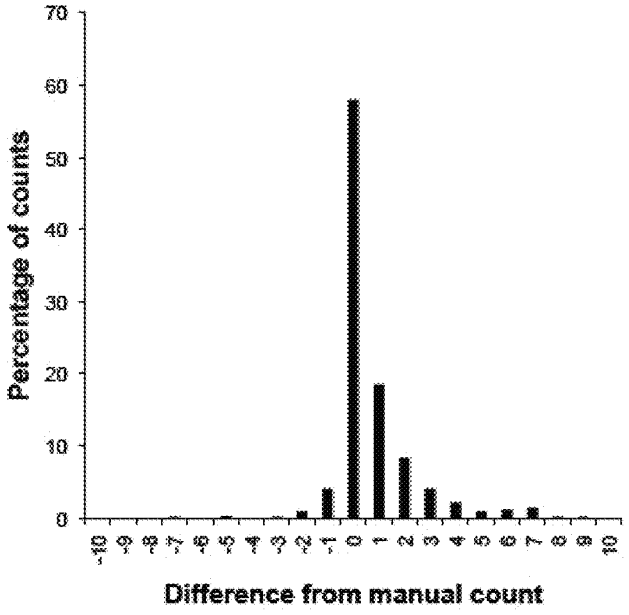

Average Fluorescence Intensity (AU)
34,000
33,900
33,800
33,700
33,600
33,500
33,400
33,300
33,200
33,100
33,000
4x Array Volume
0
10
20
30
40
Perfused Volume (µl)

FIGURE 19

20a



20b



FIGURES 20a-b

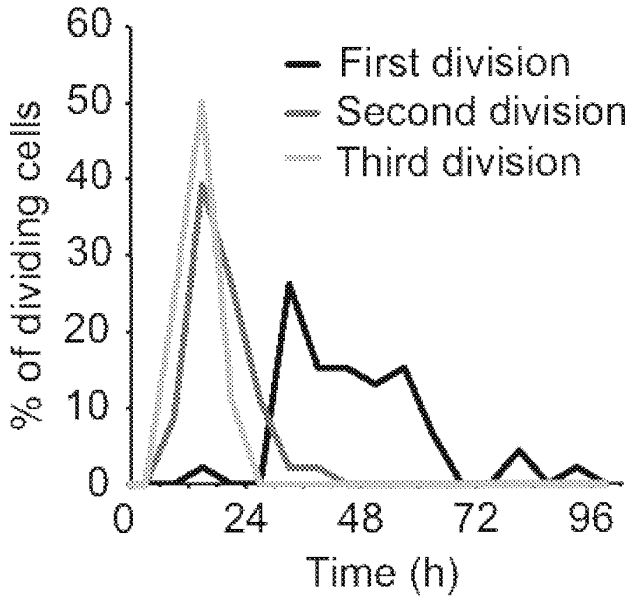

60
50
40
30
20
10
0
% of dividing cells
First division
Second division
Third division
0
24
48
72
96
Time (h)

FIGURE 21

SYSTEM AND METHOD FOR MICROFLUIDIC CELL CULTURE

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Patent Application Ser. No. 61/362,213 entitled "SYSTEM AND METHOD FOR MICROFLUIDIC CELL CULTURE" filed on 7 Jul. 2010, which is incorporated herein by reference in its entirety.

BACKGROUND

1. Field

This invention relates to microfluidic devices. In particular, the invention relates to microfluidic devices and their uses and methods for culturing cells for extended periods of time.

2. Description of Related Art

Cell population heterogeneity poses a major obstacle to understanding complex processes that govern tissue-specific cellular responses, differentiation, and disease development. Averaged measurements of large numbers of cells often obscure the variable responses of individual or rare cells. New technologies for studying cellular heterogeneity at the single cell level under well-defined chemical environments are therefore of great interest in the study of cells (for example, stem cell fields).

The need for scalable single cell analysis is particularly acute in the study of hematopoietic stem cell (HSCs) growth and differentiation. The analyses of clonal cultures derived from single HSCs have been performed for a number of years and these have already provided some insights into the proliferation kinetics of the input cells, their in vitro responses to varying growth factor conditions, and their rapid loss ex vivo of the differentiation pattern that is typically preserved when they expand in vivo. Such experiments have shown that quiescence and delayed cell cycle entry correlate with higher potency (Brummendorf, T H. et al. J. Exp. Med. 188, 1117-1124 (1998); Audet, J. et al. Biotechnol. Bioeng. 80, 393-404 (2002)), that asymmetric cell divisions are features of HSCs with long-term hematopoietic activity (Ma, N. N. et al. Biotechnology and Bioengineering 80, 428-437 (2002)), and that the probability of HSCs executing a self-renewal decision in vitro is regulated by the types and concentrations of growth factors to which it is exposed (Ma, N. N. et al. Biotechnology and Bioengineering 80, 428-437 (2002); Pineault, N. et al. *Leukemia* 19, 636-643 (2005); Pineault, N. et al. *Molecular and Cellular Biology* 24, 1907-1917 (2004)). Recently, the study of HSCs using automated time-lapse imaging and, in some cases, micropatterned substrates, has enabled increased time resolution and the identification of new phenotypes associated with particular biological behaviors (Audet, J. et al. Biotechnol. Bioeng. 80, 393-404 (2002); El-Ali, J. et al. Nature 442 , 403-411 (2006); Faley, S. L. et al. Lab Chip 9, 2659-2664 (2009); Wang, Z. H. et al. Lab Chip 7, 740-745 (2007); Figallo, E. et al. Lab Chip 7, 710-719 (2007)). These latter approaches indicate the power of higher throughput micro-culture systems, even though they lack desirable features including variable schedules of medium exchange.

Integrated microfluidic systems provide many advantages for live-cell microscopy tracking studies. These advantages include low reagent consumption, precise temporal control over growth conditions, and an ability to work with but not

be limited to small numbers of input cells. Although these advantages have been well explored to analyze yeast and bacterial cell responses (Balagadde, F. K. et al. Science 309, 137-140 (2005); Taylor, R. J. et al. Proc. Natl. Acad. Sci. USA 106, 3758-3763 (2009)), applications to mammalian cells are less developed. Whereas fluid- and cell-handling capabilities have been well established (El-Ali, J. et al. Nature 442 , 403-411 (2006)), there have been relatively few reports of the application of programmable microfluidic systems to the long-term analysis of biological responses presumably owing to the difficulties in obtaining robust growth of mammalian cells in microfluidic devices. Previous mammalian microfluidic culture systems have been largely restricted to experiments with adherent cells incubated for short periods of time (hours) (Faley, S. L. et al. Lab Chip 9, 2659-2664 (2009); Wang, Z. H. et al. Lab Chip 7, 740-745 (2007)) in relatively large volumes of medium (Figallo, E. et al. Lab Chip 7, 710-719 (2007)) and/or maintained under high perfusion rates (Kim, L. et al. Lab Chip 6, 394-406 (2006); Korin, N. et al. Biomed. Microdevices 11, 87-94 (2009)). With a few notable exceptions (Lee, P. J. et al. Biotechnol. Bioeng. 94, 5-14 (2006); Hung, P. J. et al. Biotechnol. Bioeng. 89(1) (2005)), longer-term microfluidic mammalian cell culture has been characterized by reduced growth rates and even deviations from normal phenotypes (Korin, N. et al. Biomed. Microdevices 11, 87-94 (2009); Paguirigan, A. L. & Beebe, D. J. Integr. Biol. 1, 182-195 (2009)). Technical hurdles in available devices include dehydration, immobilization of nonadherent cells to facilitate medium exchange and recovery of the cultured cells for subsequent phenotypic or functional analysis. Furthermore, a microfluidic cell culture system that achieves culture conditions similar to those obtained in standard macrocultures, and allows for analysis of heterogeneous cell behaviour to generate differentiated cells both in vitro and in vivo would have practical utility.

Microfluidic devices made of polydimethylsiloxane (PDMS), a transparent and biocompatible silicone elastomer, have been widely used for cell-culture applications and provide high gas permeability for the efficient exchange of oxygen and carbon dioxide. However, PDMS is also permeable to some small molecules (Berthier, E. et al. Lab Chip 8, 852-859 (2008); Regehr, K. J. et al. Lab Chip 9, 2132-2139 (2009)) and allows for rapid transport of water vapor, which may result in dehydration (Heo, Y. S. et al. Anal. Chem. 79, 1126-1134 (2007); Hansen, C. L. G. et al. J. Am. Chem. Soc. 12 8, 3142-3143 (2006)). The high surface-to-volume ratios characteristic of nano-volume culture chambers further promote dehydration of microfluidic devices. In addition, small hydrophobic molecules can diffuse in the elastomeric material and be depleted from the medium. These variations may lead to spurious biological responses, reduced growth rates and even cell death.

SUMMARY

In a first embodiment, there is provided a method of culturing a cell, the method including: (a) retaining the cell at a retaining position within a chamber having a chamber volume; and (b) flowing a perfusing fluid through the chamber, wherein the perfusing fluid enters the chamber through an inlet at an inlet position and exits the chamber through an outlet at an outlet position, wherein the perfusing fluid has a greater velocity laminar flow adjacent the inlet and outlet positions than at the retaining position, and wherein a first region of the chamber is spaced apart from

the retaining position and where the first region is interposed directly between the inlet and outlet positions.

In a further embodiment, there is provided a method of culturing a cell, the method including: (a) retaining the cell at a retaining position within a chamber; (b) flowing a perfusion fluid into the chamber through an inlet; and (c) flowing the perfusion fluid out of the chamber through an outlet wherein the outlet is positioned such that gravitational forces acting on the cell to keep it at or near the retaining position exceed hydrodynamic forces acting on the cell to move it toward the outlet.

In a further embodiment, there is provided a method of culturing a cell, the method including: retaining the cell in a volume of perfusion fluid, wherein the volume is less than about 10 nL; and placing the volume of perfusion fluid in fluid communication with a reservoir fluid, wherein the reservoir fluid has a volume greater than the volume of perfusion fluid.

The first region of the chamber may be defined as the volume of the chamber interposed directly between the inlet and outlet positions. Furthermore, the first region is spaced apart from the retaining position such that the velocity of the perfusion fluid at the retaining position is lower than the velocity of the perfusion fluid adjacent the inlet and outlet positions during perfusion. Similarly, the first region is spaced apart from the retaining position such that the velocity of the perfusion fluid at the retaining position is lower than the velocity of the perfusion fluid within the first region during perfusion. Accordingly, the velocity of the perfusion fluid around a cell at the retaining position may be regulated such that the velocity of the perfusion fluid is lower at the retaining position than adjacent the inlet and outlet positions during perfusion. The velocity of the perfusion fluid around a cell at the retaining position may be regulated such that the velocity of the perfusion fluid is lower at the retaining position than the first region. The speed of the perfusion fluid may be decreased to less than 50 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 40 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 30 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 20 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 10 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 5 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 4 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 3 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 2 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to less than 1 μm/s as the perfusion fluid approaches the retaining position. The speed of the perfusion fluid may be decreased to 0 μm/s as the perfusion fluid approaches the retaining position.

The chamber may have a top and a bottom, and the retaining position may be at the bottom. The inlet position may be proximal to the top. The outlet position may be proximal to the top. The cell may be retained at the bottom by gravitational forces. The method may further include regulating osmolarity of the perfusion fluid within the chamber. The regulating osmolarity of the perfusion fluid within the chamber may include placing the chamber in fluid communication with a bathing fluid, wherein the bathing fluid has a volume greater than the chamber volume. The regulating osmolarity of the perfusion fluid within the chamber may include placing the chamber in gaseous communication with a bathing fluid, wherein the bathing fluid has a volume greater than the chamber volume. The bathing fluid and the perfusion fluid may be iso-osmotic. The cell may be a suspension cell. The perfusion fluid may include any one or more of: a cell culture medium; an immunostaining agent; an enzymatic reagent; a dye; an oil; and a bead-containing solution.

The length of the first region may be less than or equal to a length of the shortest distance between the retaining position and the first region. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 3:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 2:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 3:2. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 1:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.5. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.6. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.7. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.8. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.9. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 1.0. The method may further include flowing the cell into the chamber prior to retaining the cell at the retaining position. The method may further include isolating a clone of the cell. The method may further include tracking the progeny of the cell.

The flow of the perfusing fluid may be intermittent. The flow of the perfusing fluid may be continuous. The flow of the perfusing fluid may be intermittent. The flowing of the perfusing fluid may be continuous. The replenishing of the perfusing fluid may be intermittent. The replenishing of the perfusing fluid may be continuous.

A value for x may be less than or equal to the value for y, wherein x is the length of the shortest distance between the inlet and the outlet and y is the length of shortest distance between the retaining position a region of the chamber that is interposed directly between the inlet and outlet positions. The ratio of x:y of the chamber is greater than 0.5.

The method may further include replenishing the perfusion fluid. The perfusion fluid and reservoir fluid may be iso-osmotic.

In a further embodiment, there is provided a microfluidic device for perfusing a cell with perfusion fluid, the device including: a chamber, having: (i) at least one inlet; (ii) at least one outlet; and (iii) a cell retainer; wherein the inlet and the outlet are in fluid communication with the cell retainer, and wherein the outlet is positioned such that, when the device is being perfused, gravitational forces acting on the cell to keep it at or near the retainer exceed hydrodynamic forces acting on the cell to move it toward the outlet.

In a further embodiment, there is provided a microfluidic device for perfusing a cell with perfusion fluid, the device including: a chamber, having: (i) at least one inlet; (ii) at least one outlet; and (iii) a cell retainer; wherein the inlet and the outlet are in fluid communication with the cell retainer; and wherein a first region of the chamber is interposed directly between the inlet and outlet positions and is spaced apart from the cell retainer.

The microfluidic device may further include an osmolarity regulator for regulating the osmolarity of the perfusion fluid. The osmolarity regulator may include an iso-osmotic reservoir in fluid communication with the chamber. The microfluidic device may further include a reservoir for holding a reservoir fluid, wherein the reservoir is in fluid communication with the chamber. The reservoir fluid may be iso-osmotic with the perfusion fluid. The microfluidic device may further include flow channels in fluid communication with the chamber via the at least one inlet and the at least one outlet. The chamber may have a top and a bottom, and the cell retainer is at the bottom. The inlet may be proximal to the top. The outlet may be proximal to the top. The cell may be a suspension cell. The perfusion fluid may include any one or more of: a cell culture medium; an immunostaining agent; an enzymatic reagent; a dye; an oil; and a bead-containing medium. The distance between the inlet and the outlet may be less than a distance between the cell retainer and the outlet. The ratio of the length of the distance between the inlet and the outlet to the distance between the cell retainer and the outlet may be less than 2:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 3:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 2:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 3:2. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be less than 1:1. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.5. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.6. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.7. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.8. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 0.9. The ratio of the length of the first region to the shortest distance between the retaining position and the first region may be more than 1.0.

The device may be an array of at least 100 chambers. The chambers may be connected in parallel. The chambers may be serially connected. The chambers may be connected in parallel and in series. The chambers may be connected partially in parallel and partially in series. The microfluidic device may be operable to be perfused intermittently. The microfluidic device may be operable to be perfused continuously.

In a further embodiment, there is provided a use of a microfluidic device described herein for tracking progeny of the cell.

In a further embodiment, there is provided a use of a microfluidic device described herein for selection of a clone of the cell.

In a further embodiment, there is provided a use of a microfluidic device described herein for observing cell-cell interactions.

In a further embodiment, there is provided a use of a microfluidic device described herein for observing autocrine effects of the cell.

The selection may be based on the amount of a factor produced by the cell or the clone. The factor may be a protein. The factor may be a nucleic acid. The cell may be a suspension cell. The use may further include recovery of the cell or clone thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

In drawings which illustrate embodiments of the invention.

FIG. **1** is a top view of a microfluidic device with two expanded views (11×12 chamber view (size reference bar=1 mm) and a 4 chamber view (size reference bar=100 μm)) to magnify details of the microfluidic device according to an embodiment.

FIG. **2** is an exploded oblique view of a portion of the microfluidic device depicted in FIG. **1**, showing the various layers associated with an individual microfluidic device.

FIG. **3** is an oblique view from below of 4 microfluidic chambers depicted in FIG. **1** with associated fluid channels and control layers.

FIG. **4** is a cross-sectional view of a chamber and a channel of the microfluidic device depicted in FIG. **1**, showing the dimensions and volume of the chamber, and a depiction of fluid speed in the control layer and chamber while the chamber is being perfused.

FIG. **5** is an oblique view of the microfluidic device (array) depicted in FIG. **1**, further showing the microfluidic device containing an iso-osmotic bath pressurized by a syringe, the inlet and outlet, and the control lines (pumps and valves) which may be connected to solenoid actuators (not shown).

FIG. **6** shows differences in cell survival during microfluidic culture in the indicated conditions compared to the high [SF] condition, wherein the cells were imaged every 12 min, and survival curves were normalized to a third-order polynomial fit for the high [SF] conditions.

FIG. **7** shows cumulative division kinetics of primary HSCs that are cycling (excluding dead and quiescent cells) in the indicated in vitro conditions for the first and second divisions.

FIG. **8** shows individual growth curves of primary murine HSCs under different Steel factor (SF) exposure conditions, where the growth curves were generated using the enhanced bifocal image analysis algorithm and the analysis was started after 21 hours to allow small quiescent cells to reach a suitable size for detection by image analysis.

FIG. **9***a* is a scatter plot depicting cell counts before and after medium exchange (2 μL/min for 10 minutes) showing minor variations attributable to cell division and cell death.

FIG. **9***b* is a graph depicting the efficiency of cell recovery from chambers by cell count in individual chambers and recounting of cells successfully transferred to a well, showing that on average 91% of the cells from individual colonies of different sizes could be recovered.

FIG. **9***c* is a graph depicting lineage tracking of cells for 3 clones following manual inspection of images, where the cells were imaged every 5 minutes and media was exchanged every 6 hrs.

FIG. **10** is a graph comparing the of average growth rates of ND13 cells in 24-well dish culture, single cell 96-well

culture, a microfluidic device as described herein with and without an iso-osmotic reservoir (arrows show growth medium exchange).

FIG. **11** is a graph of cell concentration (1 cell, 2-15 cells, 16-30 cells, 31-45 cells, and >45 cells) over time for various seeding densities grown in a microfluidic device according to an embodiment, but with no medium exchange and no iso-osmotic bath. This Figure shows a strong inverse correlation with the initial number of cells in each chamber.

FIG. **12***a* are time-lapse images of clonal expansion in a chamber of a microfluidic device according to an embodiment (at 0 hr and at 6 hr intervals thereafter until 66 hrs.).

FIG. **12***b* is a histogram depicting distribution of average doubling times of clonal ND13 cultures up to 72 hrs. in a microfluidic device according to an embodiment, showing that the cells growth rates are highly heterogeneous, whereby only a small fraction of fast growing cells contributed to the overall growth rate, while 52% of the cells did not give rise to colonies (i.e. cells marked 'no proliferation' (52%) did not divide during this period or died).

FIG. **12***c* is a graph depicting the proliferation profile of individual clones over time (as counted by automated image analysis for individual ND13 cells) in a microfluidic device according to an embodiment, also showing highly variable growth rates.

FIG. **13***a* is a graph depicting the proliferation of lin^+ clones in ND13 cells grown in a microfluidic device according to an embodiment, wherein the majority of the lin^+ cells either died or did not give rise to colonies.

FIG. **13***b* is a graph depicting the proliferation of lin^- clones in a microfluidic device according to an embodiment, wherein the lin^- cells gave rise to colonies of different sizes.

FIG. **13***c* is an image depicting live immunostaining of small clonal populations in a microfluidic device according to an embodiment, wherein Nup98-HOXD13 clones were stained for lineage markers B220, Gr-1, and Mac-1 at 0 Hrs. and after 72 hours inside the microfluidic device.

FIG. **13***d* is a histogram depicting the progeny of Nup98-HOXD13 clones taken from a microfluidic device according to an embodiment and a control plate to compare colony forming cells (CFC—colonies/100 cells) using methylcellulose assays.

FIG. **14***a* is a schematic diagram of a study to compare primary hematopoietic stem cells (HSC) activity in NUP98-HOXA10hd (NA10dh)-transduced hematopoietic populations cultured in a microfluidic device according to an embodiment as compared to hematopoietic populations cultured in a macroscale 96-well plate as compared by competitive repopulating cell (CRU) assay.

FIG. **14***b* shows a comparison of macroscale 96-well plate cultured cells (control) with microarray cultured cells, whereby NUP98-HOXA10hd cells maintained functional HSC activity after being cultured in the microfluidic array according to an embodiment and were able to reconstitute the blood-forming system of lethally irradiated mice.

FIG. **15***a* is a histogram depicting the ability of NA10 hd hematopoietic populations cultured in a microfluidic device according to an embodiment and hematopoietic populations cultured in a macroscale 96-well plate to produce myeloid and lymphoid lineages as defined by lineage markers Gr-1/Mac-1, B220, and CD4/CD8.

FIG. **15***b* is a histogram depicting distribution of average doubling times of single HSC cells (NA10hd) in a microfluidic device according to an embodiment after being transduced with NA10 hd (apparent doubling time 13 hrs).

FIG. **16** shows a microfluidic cell culture array for temporal stimulation and parallelization of experiments, wherein the microfluidic cell culture array contains 6,144 chambers and can support up to 8 different conditions simultaneously, but only the top half of the array was used to study murine HSCs due to the relatively small cell numbers and 6 different conditions were distributed across the array as shown.

FIG. **17***a* shows representative images from an automated image analysis algorithm for cell quantification of cells in a chamber of a microfluidic device according to an embodiment, where segmentation was accomplished through three main steps: chamber segmentation (A-E), cell-containing region segmentation (F-J), and then single cell isolation (K-O). First, the individual chambers are segmented from the image background.

FIG. **17***b* shows a plot comparing automated image analysis depicted in FIG. **17***a* and manual cell counts, wherein the straight line represents the 1:1 slope.

FIG. **18** shows a mature myeloid population derived from lineage negative ND13 cells, where the ND13 cells were stained for Gr-1, Mac-1 and B220 and sorted by flow cytometry and the lin^- fraction was cultured for 9 days and gave rise to a new lin^+ population.

FIG. **19** is a graph of average fluorescence intensity as a function of perfused volume.

FIG. **20***a* is a scatter plot comparing the results of manual cell counts and automated cell counts employing a bifocal algorithm

FIG. **20***b* is a histogram showing absolute differences between the algorithm and manual counts.

FIG. **21** shows a percentage of dividing cells over time for mouse HSCs cultured in the microfluidic array under high SF concentration (300 ng ml-1), where single cells were imaged every 4 min, and the times for the first, second and third divisions were identified by manual inspections of the videos for 46 cells.

DETAILED DESCRIPTION

Definitions

A "microfluidic device", as used herein, refers to any device that allows for the precise control and manipulation of fluids that are geometrically constrained to structures in which at least one dimension (width, length, height) may be less than 1 mm.

"Perfusion" or "perfusing", as used herein, refers to the passage of fluid, such as culture media, over cells for the purposes of nutritive delivery and waste removal. A person skilled in the art will understand that the fluid does not necessarily flow over the cell, but may ultimately arrive at a cell by the process of diffusion. Furthermore, perfusion or perfusing of a chamber or the microfluidic device as a whole may be continual or intermittent provided that the fluid exchange provides sufficient nutrient delivery and/or waste removal and or other factors or reagents to keep the cells viable (if that is the desired result) and/or to maintain desired conditions for the particular cell and/or assay as desired.

A "perfusion fluid", as used herein, refers to any fluid with which a cell in a chamber is perfused. A person skilled in the art will understand that a perfusion fluid may comprise factors or reagents with which it is desired to present to the cell. Factors or reagents may include proteins (e.g. interferon-α, TAT, fibronectin, bovine serum albumin), small molecules (e.g. all-trans retinoic acid, imatinib), growth factors (e.g. IL-3, IL-6, IL-11, SCF, GM-CSF), immunostaining agents (e.g. monoclonal antibodies, polyclonal antibodies, fluorophores, blocking solution), enzymatic reagents (e.g. horseradish peroxidase, luminol), oils (e.g. mineral oil,

fluorinated oil), dyes (e.g. rhodamine, fluorescein, Hoescht, functionalized beads (e.g., magnetic beads, polymer beads, protein A-coated beads, protein G-coated beads), buffers (e.g. PBS, Hank's balanced solution, HEPES), PCR solutions (e.g. polymerase, nucleic acids, primers), cell transfection solutions (e.g. fibronectin, retronectin, polyethylenimine), cell fixation solutions (e.g. ethanol, formaldehyde), miRNAs, siRNAs, molecular beacons, amino acids (e.g. glutamine), antigens, semi-solid matrix (e.g. methylcellulose, Matrigel®), etc.

A "chamber" or "cell capture chamber", as used herein, refers to an enclosed space within a microfluidic device in which one or more cells may be isolated from a larger population of cells as the cells are flowed through the device. Each chamber will have at least one inlet for permitting fluid, including fluid containing cells, to enter the chamber, and at least one outlet to permit fluid to exit the chamber. Persons skilled in the art will understand that an inlet or an outlet can vary considerably in terms of structure and dimension, and may be reversibly switched between an open position, to permit fluid to flow into or out of the chamber, and a closed position to seal the chamber and thereby isolate and retain its contents, whereby the aperture may also be intermediate between the open and closed positions to allow some fluid flow. Each chamber will further have at least one cell retaining position which may comprise at least one cell retainer.

The direction of fluid flow through the chamber dictates an "upstream" and a "downstream" orientation of the chamber. Accordingly, an inlet will be located at an upstream position of the chamber, and an outlet will be generally located at a downstream position of the chamber. It will be appreciated by a person of skill in the art, that the designation of an "inlet" or an "outlet" may be changed by reversing the flow within the device or by opening one or more alternative aperture(s).

A "cell retaining position" or "retainer position", as used herein, refers to a location in the chamber at which a cell is maintained during cell culture and media exchange. A retaining position may include at least one cell retainer. According to some embodiments, the retaining position may be a determined position within the chamber. However, a person skilled in the art will understand that a retaining position may comprise a zone within the chamber. The important characteristic of a retaining position is that hydrodynamic forces are insufficient to facilitate the escape of a cell through the outlet while the cell is in the retaining position and shear forces on the cell, if any, do not damage the cell. Depending on the cell type, the cell may adhere, either weakly or strongly, to the cell retaining position or a cell retainer or may be held in place by gravitational forces or a cell trap.

A "cell retainer", as used herein, refers to any structure which serves to maintain a cell within a retaining position. In a simple embodiment, the cell retainer may be the bottom of the chamber and the cells may be held in place by gravitational forces. Alternatively, a cell retainer may include a cell trap positioned to receive (and retain) a cell that is flowed into the chamber. Furthermore, a substrate may be provided at the cell retaining position that facilitates retention of the cells. For example, extracellular matrix (ECM) components or integrin or functionalized beads etc. may be deposited on at the retaining position to facilitate retention of cells.

An "inlet" or an "outlet", as used herein, may include any aperture whereby fluid flow is restricted through the inlet or outlet. There may be one or more valves to control flow, or flow may be controlled by separating the fluid channels, which lead to the inlets and outlets with a layer which prevents flow (for example, a control layer or isolation layer). Alternatively, flow may be regulated by the rate at which passed through the device. An "inlet position", as used herein, refers to a position in the chamber where an inlet is located. Similarly, an "outlet position", as used herein, refers to a position within a chamber where an outlet is located. According to embodiments, the inlet position, outlet position, and retaining position will not be co-linear.

A "first region", as used herein, refers to a region of the chamber that is interposed directly between the inlet and outlet positions. In some embodiments the first region is spaced apart from the retaining position. According to some embodiments described herein, the first region may be generally at the top of the chamber and the cell retaining position may be generally at the bottom of the chamber, such that the velocity of the fluid in the cell retaining position is slower than the velocity of the fluid in and around. In some embodiments the velocity of fluid in cell retaining position is or approaches zero and the only perfusion fluid that enters the cell retaining position is by diffusion or convection, thereby providing fresh diffusion fluids to the cell. According to some embodiments described herein, the speed of the fluid flow in the first region will be sufficiently low such that the hydrodynamic forces of the fluid urging a cell from the retaining position to an outlet are exceeded by forces, e.g. gravitational forces, urging the cell toward the cell retaining position.

A "cell trap", as used herein, refers generally to a means for receiving and retaining cells at a pre-determined location over time. A cell trap may comprise localized surface modifications for chemical immobilization of a cell. Alternatively, the cell trap may be a mechanical trap, a hydrodynamic trap (Skelley, A M et al. Nat Methods 6(2):147-152 (2009); Li, P. C. H. et al. Lab on a Chip 4, 174-180 (2004); Li, X. & Li, P. C. H. On-Chip Dye Loading, Cell Contraction by Chemical Stimulation, and Quantitative Fluorescent Analysis of Intracellular Calcium. Anal. Chem. 77, 4315-4322, doi:10.1021/ac048240a (2005); Di Carlo, D. et al. Anal. Chem. 78, 4925-4930, doi:10.1021/ac060541s (2006)), a hydrodynamic balancing trap (Rowat, A. C. et al. Proceedings of the National Academy of Sciences 106, 18149-18154, doi:10.1073/pnas.0903163106 (2009); and Kobel, S. et al. Lab on a Chip 10, 857-863 (2010)), an active valving trap (Warren L, et al. Proc Natl Acad Sci USA 103(47):17807-17812 (2006); Skelley, A M et al. Nat Methods 6(2):147-152 (2009); Li, P. C. H. et al. Lab on a Chip 4, 174-180 (2004); King, K. R. et al. Lab on a Chip 7, 77-85 (2007); Marcy, Y. et al. Proc. Natl. Acad. Sci. U.S.A. 104, 11889-11894 (2007)), a dielectrophoretic trap (Voldman, J. et al. Anal. Chem. 74, 3984-3990, doi:10.1021/ac0256235 (2002)), a DNA immobilization trap (Toriello N M, et al. Proc Natl Acad Sci USA 105(51):20173-20178 (2008)), a gel encapsulation trap (Braschler, T. et al. Lab on a Chip 5, 553-559 (2005)), a magnetic trap, an acoustic trap or an optical trap (Neuman, K. C. et al. Biophys. J. 77, 2856-2863 (1999)). In various embodiments described herein, a cell trap will generally be positioned directly in the path of the smaller cross sectional of cell flow created by the funnel. Where a mechanical funnel is used according to various embodiments described herein, a trap may be positioned directly after the downstream opening of the funnel. Furthermore, additional cell trapping and funneling methods may be found in PCT/CA2011/000612.

A "mechanical trap", as used herein, refers to a physical cell trap such as a cage.

A "hydrodynamic trap", as used herein, refers to a cell trap in which the force of the fluid in motion plays a role in retaining a trapped cell in its position. A hydrodynamic trap may be also be comprised of a mechanical trap in which a cell is captured and retained. Exemplary mechanical traps are described in PCT/CA2011/000612. In certain embodiments hydrodynamic traps may be utilized. However, it may be desirable to have three or more inlets to the cell capture chamber so that the flows may be adjusted in order to direct cells to the traps.

A "dielectrophoretic trap", as used herein, refers to a cell trap in which cells, being dielectric objects, are retained by the forces generated by a non-uniform electric field.

A "magnetic trap", as used herein, refers to a cell trap employing magnetic fields to retain cells. Typically, cells will be labeled with magnetic particles, and then positioned and retained by the magnetic fields. However, magnetic traps can also be used to trap-non-magnetic cells in suitable buffers.

An "acoustic trap", as used herein, refers to a cell trap in which ultrasonic standing waves are used to generate stationary pressure gradients that exert forces that position and retain cells.

An "optical trap", as used herein, refers to a cell trap in which a tightly focused laser beam, typically a near-infra red laser beam, is used to draw cells in the direction of the beam.

The size of the cell trap may be varied according to the size, type, mechanical properties, or number of cells that are desired to be trapped. A microfluidic device according to various embodiments may further include a combination of trap designs for the capture of a range of cell types. Furthermore, each chamber could include multiple traps or each chamber or a subset of chambers may be optimized to capture a particular cell type. In such embodiments, the frequency of cells of a particular size or having particular characteristics that are trapped may be used for diagnostic or other assay purposes. Alternatively, the contents of a group of cells caught in a single trap may be processed and analyzed.

A chamber may further include cell funnel, and a "cell funnel" as used herein, refers to an apparatus which is designed to focus the flow of cells from a first location, where the cells are dispersed, to one or more desired second or more locations within the chamber wherein the cell funnel has a smaller cross sectional area of cell flow. The cell funnel may exert a force to direct cells towards the one or more desired locations within the cell capture chamber. For the purposes of clarity, "force" is defined herein as any influence that causes a free body (e.g. a cell) to undergo a change in velocity. Funnels may either span the entire height and/or width of the cell capture chamber, or partially span the height and/or width. Exemplary cell funnels are described in PCT/CA2011/000612.

A "bathing fluid", as used herein, refers to any fluid which is used to regulate the osmolarity of fluid, e.g. perfusion fluid, within a chamber. A bathing fluid may be iso-osmotic with the perfusion fluid or sufficiently close to iso-osmotic such that the osmolarity of the fluid remains in a range that is suitable for cell culturing. According to an embodiment described herein, bathing fluid will generally be present in a volume greater than a chamber. The bathing fluid may be a in a reservoir that is in gaseous communication with the chamber. For example, the reservoir may be separated from the chamber by a gas-permeable PDMS membrane, wherein the water exchange occurs in vapor phase.

An "osmolarity regulator" is a system for regulating the osmolarity of the perfusion fluid within a chamber and/or a microfluidic device as a whole. For example, the osmolarity regulator may comprise an iso-osmotic reservoir or bath in fluid communication with the chamber, wherein the iso-osmotic reservoir is filled with or resupplied with a bathing fluid that may be iso-osmotic with the perfusion fluid. The term bath and reservoir are used interchangeably with regards to osmolarity regulation of the device.

Alternatively, osmolarity may be regulated in the chambers by immersing the chambers in a large volume media bath that would be recirculated to maintain osmolarity. Alternatively, the osmolarity may be regulated in the chambers by enclosing the microfluidic device in an environmentally regulated enclosure.

"Aspect ratio", as used herein, refers to the ratio (y:x) of the shortest distance between the cell retaining position and the first region (y) to the length of the first region (x). In various embodiments where the inlet and outlet is at the top of the chamber, such that the first region is horizontal and defines an area that is interposed directly between the inlet and outlet positions, the aspect ratio will effectively been the ratio of the height of the chamber (minus the height of the first region) to the width of the chamber.

A "hydrodynamic force", as used herein, refers to a force exerted by a fluid in motion.

An "adherent cell", as used herein, refers to a cell which requires contact with a surface for growth or proliferation in vitro. For example, SAOS-2; U-2 OS; U-2 OS CycE; A172; T98G; U373MG; U87MG; SVGp12; BT-474; HMEC; MCF-7; MCF-10A; MDA-MB-231; MDA-MB-231M; MDA-MB-436; MDA-MB-468; SK-BR-3; T47D; ZR-75-1; MA11; PM1; SUM1315mo2; JIMT-1; HCC-1937; KPL-4; SUM-102PT; HeLa; HCT-116; SW480R18; SW480; HT29; Caco-2; SW620; DLD-1; LS174T; SW48; RKO; HCT-15; LS1034; AGS; CHO; SVpgC2a; HEK293; HEK293TREX; HA1ER; HA1EB; A549; A549EpoB40; U1690; A549EpoB480; NCI-H460; MDA-MB-435; UACC-257; NIH3T3; 1A9; 1A9/PTX10; 1A9/PTX22; Ascites cells; KF28; KF28Tx; KFr13; KFr13Tx; Primary ovarian solid tumor cells; OVCAR-3; OVCAR-4; OVCAR-5; OVCAR-8; OVCAR-8/ADR; SU.86.86; CAPAN-1; Hs 766T; 22-RV-1; DuCaP; LAPC-4; LnCaP; Primary prostate stromal cells; Primary prostate epithelial cells; MDA-P; CA-1; MDA-PCA-2b; PC-3; PC-3M; PWR-1E; RWPE-1; VCaP; WPE-1/NA22; WPE-1/NB11; WPM4-1; P97E; ALVA-31; RD; and A431. Generally, most cells derived from solid tissues are adherent cells (Rantala, J. K. et al. BMC Genomics 12:162 doi:10.1186/1471-2164-12-162 (2011)).

A "suspension culture", as used herein, refers to a culture in which cells grow or multiply while suspended in a suitable fluid medium. Accordingly, a "suspension cell", as used herein, refers to any cell which is cultured while suspended in a suitable medium. A person skilled in the art will understand that a suspension cell need not naturally exist or multiply while suspended in a fluid medium, provided that the cell is adapted to grow or survive in suspension culture. Furthermore, a person skilled in the art will understand that, while a suspension cell is generally non-adherent, a suspension cell may retain some ability to adhere to a surface while being cultured in suspension. Accordingly, adherent or weakly adherent cells may be cultured as suspension cells under appropriate conditions. Suspension cells may include, for example, Chinese Hamster Ovary (CHO) cells; K562; BAF3; HEK293; Sf21; Sf9; S2; primary bone marrow or bone marrow-derived cells; primary cord blood cells, primary hematopoietic cells, primary hematopoietic stem cells; hybridoma cells or primary blood-

born cancer cells. Suspension cells may be hematopoietic in origin or may be adapted to suspension culture from an adherent cell line.

A "fluid injection channel", as used herein, refers to any conduit through which fluid may be introduced into a chamber of the device. A fluid injection channel can be used to deliver any fluid to a chamber including cell suspensions, cell culture media, wash buffers, reaction mixes, factors, reagents, functionalized beads, etc.

An "auxiliary chamber", as used herein, refers to any chamber subsidiary to a cell capture chamber. Auxiliary chamber can be used for treatment or assaying of a captured cell, or its isolated contents. Treatment can include cell preparation steps including culture, washing, lysis, and fractionation. Assaying may include DNA and RNA amplification and detection, including mitochondrial PCR; genomic PCR; digital PCR, RT-PCR, RTq-PCR, multiple displacement amplification (DNA), rolling circle amplification sequencing, degenerate PCR, molecular inversion probes, molecular beacons, as well as other DNA/RNA amplification and detection methods, in vitro transcription, ligation, immunochemistry; reporter expression analysis; hybridization studies; and so forth. Several auxiliary chambers may be connected, in tandem and/or in parallel, to a single cell capture chamber, such that multiple treatments may be performed on the contents of a single cell capture chamber. A valve may be positioned between an auxiliary chamber and the cell capture chamber, or between auxiliary chambers, to regulated fluid flow between chambers.

Methods

The following methods were used for the fabrication of embodiments described herein in the examples disclosed below. It will be apparent that other methods, materials and designs are possible for creating other embodiments while remaining within the spirit of the invention described herein.

Microfluidic Cell Culture Array Fabrication

Devices were entirely made out of PDMS (Sylgard 184®, Dow Corning™). The cell culture array, control, and membrane layers were assembled using multilayer soft lithography techniques (Unger, M. A. et al. Science 288, 113-116 (2000); and Thorsen, T. et al. Science 298, 580-584 (2002).) while the iso-osmotic bath and cover layers were integrated by PDMS stamping (Satyanarayana, S. et al. J. Microelectromech. Syst. 1414, 392-399 (2005)). Chips were covalently bound to glass slides by oxygen plasma treatment. Devices were left at 80° C. for at least 5 days and autoclaved prior to use for cell culture applications to drive the curing reaction towards completion. Detailed protocols for mold and device fabrication are as follows.

Wafer Fabrication Protocol. Each new microfluidic design is created with a drawing software such as AutoCAD. A micro-pump is located downstream of the array to avoid crushing the cells and control the speed during the loading process. Depending on the application, microfluidic cell culture arrays may contain from 1,600 to ~20,000 chambers in the order of ~4 nl each. Multiplexers, isolation valves, osmolarity regulator, hydration lines etc. can be added when necessary to offer a better control of the microenvironment. Designs are printed at 20,000 dpi on transparent masks. The fabrication of molds on a silicone substrate is performed using common photolithography techniques as described below.

Flow Wafer

Flow Channels

1. Dehydrate a wafer for 10-15 minutes at 150° C.
2. Treat the wafer with vapor phase HMDS for at least 2 minutes.
3. Pour SPR220-7.0 resist on half the diameter of the wafer.
4. Ramp at 500 rpm for 10 seconds, then spin at 1,500 rpm for 90 seconds.
5. Pre-bake the wafer at 115° C. for 120 seconds.
6. Expose for 30 s.
7. Wait 30 minutes to rehydrate the resist.
8. Develop in MF319 primary bath for around 5-10 minutes, then rinse in an MF319 secondary bath.
9. Rinse with DI water and dry the wafer with compressed nitrogen.
10. Ramp from room temperature to 190° C. and leave overnight for hard bake.

Aim: 11-13 μm after reflow

Inlet Channels

1. Pour SU8-50 resist on half the diameter of the wafer.
2. Ramp at 500 rpm for 30 seconds, then spin at 2,500 rpm for 30 seconds.
3. Soft bake the wafer for 2 minutes at 65° C., 10 minutes at 95° C., and 2 minutes at 65° C.
4. Expose for 7 s.
5. Perform a post-exposure bake for 2 minutes at 65° C., 10 minutes at 95° C., and 2 minutes at 65° C.
6. Develop in an SU8 developer primary bath for around 4 minutes, then rinse in a SU8 developer secondary bath.
7. Rinse with IPA and dry the wafer with compressed nitrogen.

Aim: 40 μm

Chambers

1. Pour SU8-100 resist on half the diameter of the wafer.
2. Ramp at 500 rpm for 10 seconds, then spin at 1,300 rpm for 50 seconds.
3. Soft bake the wafer for 5 minutes at 65° C., 70 minutes at 95° C., and 5 minutes at 65° C.
4. Expose for 25 s.
5. Perform a post-exposure bake for 5 minutes at 65° C., 18 minutes at 95° C., and 5 minutes at 65° C.
6. Develop in an SU8 developer primary bath for around 20 minutes, then rinse in a SU8 developer secondary bath.
7. Rinse with IPA and dry the wafer with compressed nitrogen.
8. Ramp up and down from room temperature to 135° C. for 20 minutes.

Aim: 160 μm

Control Wafer

1. Dehydrate a wafer for 10-15 minutes at 150° C.
2. Pour SU8-50 resist on half the diameter of the wafer.
3. Ramp at 500 rpm for 10 seconds, then spin at 4,200 rpm for 40 seconds.
4. Soft bake the wafer for 2 minutes at 65° C., 4 minutes at 95° C., and 2 minutes at 65° C.
5. Expose for 2 minutes.
6. Perform a post-exposure bake for 2 minutes at 65° C., 6 minutes at 95° C., and 2 minutes at 65° C.
7. Develop in an SU8 developer primary bath for around 2 minutes, then rinse in a SU8 developer second bath.
8. Rinse with IPA and dry the wafer with compressed nitrogen.
9. Ramp up and down from room temperature to 135° C. for 20 minutes.

Aim: 25 μm

Device Fabrication Protocol

Cleaning

1. Place control wafers in plastic box with TMCS (can clean flow wafers with PDMS, but that requires degassing) for at least 2 minutes.
2. Pour 15.0 g RTV-A and 1.5 g RTV-B (10:1 ratio) per wafer into plastic cup, place cup in mixing machine, and mix together.
3. While machine mixing, wrap 1 Petri dish per wafer with aluminum foil.
4. After mixing is done, remove wafers from TMCS box and place in Petri dishes
5. Pour PDMS onto each wafer and tilt dish so that wafer is covered with PDMS and that PDMS overflows on the foil.
6. Place in 80° C. oven for at least 20 minutes.

(Can be left overnight after performing this step.)

Flow Layer

7. Place flow wafers in plastic box with TMCS for at least 2 minutes.
8. Pour 12.5 g RTV-A and 2.5 g RTV-B per wafer in 5:1 plastic cup, place cup in mixing machine, and mix together.
9. While machine mixing, prepare aluminum wrap using metal dish.
10. After mixing is done, remove wafers from TMCS box and place in aluminum holders. Press down wafer on the bottom by folding the aluminum foil on top of wafer edges.
11. Pour PDMS onto each wafer, and level the aluminum holder with 2 micropipette tips.
12. Place into degasser machine, pressurize, and degas for until no visible bubbles are left. Prepare control layer during that time.
13. Remove from degasser and level again with 2 micropipette tips. Let sit for at least 15 min.
14. Place in 80° C. oven for 18 minutes.

Control Layer

15. Cut around cleaned wafer with surgical knife and peel off PDMS to release the cleaned wafer.
16. Place cleaned control wafer in plastic box with TMCS for at least 2 minutes.
17. Pour 15.0 g RTV-A and 0.75 g RTV-B into 20:1 plastic cup, place cup in mixing machine, and mix together.
18. Turn on gas and vacuum for spinner.
19. Ensure spinner recipe ramps in 5 seconds to 500 rpm, dwells at 500 rpm for 10 seconds, ramps to 1630 rpm in 10 seconds, dwells at 1630 rpm for 60 seconds, and ramps down to 0 rpm in 5 seconds.
20. Place wafer carefully on center of spinner chuck, close lid and secure with copper slab, and execute spinner recipe.
21. After spinning is finished, remove wafer from spinner and place in clean, new Petri dish. Let sit for at least 15 minutes.
22. Place in 80° C. oven for 18 minutes

(The control and flow layers should go into the oven at the same time.)

Membrane

23. Cut around cleaned wafer with surgical knife and peel off PDMS.
24. Pour 15.0 g RTV-A and 0.75 g RTV-B into 20:1 plastic cup, place cup in mixing machine, and mix together.
25. Turn on gas and vacuum for spinner.
26. Ensure spinner recipe ramps in 5 seconds to 500 rpm, dwells at 500 rpm for 10 seconds, ramps to 500 rpm in 10 seconds, dwells at 500 rpm for 60 seconds, and ramps down to 0 rpm in 5 seconds (A thinner membrane will result in leaky valves while a too thick membrane does not spread evenly on the wafer).
27. Place wafer carefully on center of spinner chuck, close lid and secure with copper slab, and execute spinner recipe.
28. After spinning is finished, remove wafer from spinner and place in clean, new Petri dish.
29. Let sit for at least 15 minutes and align flow/control during that time.
30. Place in 80° C. oven for 12 minutes (13 min after flow/control duo has been placed in the oven)

Flow/Control Alignment

31. Remove both flow and control wafers from the oven.
32. Cut inside the edge of the flow wafer with a surgical knife, then peel off PDMS layer from silicon wafer.
33. Place control wafer under the microscope.
34. Align flow layer to control layer, trying not to peel off and on too much.
35. Push down any bubbles that remain between the two layers, and place in 80° C. oven for 25 min.

(The blank should come out of the oven at the same time as the flow/control combo. Time out accordingly.)

Membrane/Duo Alignment

36. Remove both flow/control duo and blank wafers from the oven.
37. Cut around the edge of the control/flow wafer with a surgical knife, then peel off PDMS layer from silicon wafer
38. Place flow/control duo onto blank layer.
39. Push down any bubbles that remain between the two layers, and place in 80° C. oven for at least one hour.

(Can be left in the oven overnight after this step.)

Bath Layer

40. Pour 40.0 g RTV-A and 4.0 g RTV-B in 10:1 plastic cup, place cup in mixing machine, and mix together (This amount of PDMS gives a sufficient height to provide good support structure for inlet and outlet ports.).
41. While machine mixing, prepare aluminum wrap using metal dish.
42. Press down wafer on the bottom by folding the aluminum foil on top of wafer edges.
43. Pour PDMS onto blank wafer, and level the aluminum holder with 2 micropipette tips.
44. Place into degasser machine, pressurize, and degas until no visible bubbles are left.
45. Remove from degasser and level again with 2 micropipette tips.
46. Place in 80° C. oven for 20 minutes.

Cover Layer

47. Pour 14.0 g RTV-A and 1.4 g RTV-B in 10:1 plastic cup, place cup in mixing machine, and mix together.
48. While machine mixing, prepare aluminum wrap using metal dish.
49. Press down wafer on the bottom by folding the aluminum foil on top of wafer edges.
50. Pour PDMS onto each wafer, and level the aluminum holder with 2 micropipette tips.
51. Place into degasser machine, pressurize, and degas until no visible bubbles are left.
52. Remove from degasser and level again with 2 micropipette tips.
53. Place in 80° C. oven for 20 minutes.

Chip Assembly

54. Remove flow/control/membrane wafer, and blank wafers from the oven and let cool for about 5 minutes.
55. Dice layers into individual chips and place the chips on a ball bearing bed, flow layer down.
56. Dice the bath layer, and cut inside to create a bath having the area of the array. Leave enough space for the ports and the edges.
57. Punch holes that go in the corner of each side of the bath.
58. Dice the cover layers into pieces bigger that each chip.
59. Clean all surfaces with scotch-tape.
60. Mix together about 10.0 g RTV-A and 1.0 g RTV-B in 10:1 plastic cup, place in mixing machine, and mix together.
61. Set spinner to spin at 6,000 rpm for 6 minutes.
62. Remove blank wafer and place on spinner, pour on PDMS, and spin.
63. Remove from spinner and place in Petri dish.
64. "Stamp" the bath portion onto the liquid blank wafer and leave for 30 seconds. Make sure to stamp the right side of the bath.
65. Remove from wafer, and stick together with the flow/control portion.
66. Remove bubbles between layers.
67. Mix together about 10.0 g RTV-A and 1.0 g RTV-B in 10:1 plastic cup, place in mixing machine, and mix together.
68. Set spinner to spin at 6,000 rpm for 6 minutes.
69. Remove blank wafer and place on spinner, pour on PDMS, and spin.
70. Remove from spinner and place in Petri dish.
71. "Stamp" the cover layer portion onto the liquid blank wafer and leave for 30 seconds.
72. Remove from wafer, and stick on top of the bath portion.
73. Remove bubbles between layers
74. Leave chips to cure at room temperature overnight on ball bearings and place them in the oven.

(After this step, the chips can be left in the oven.)

Hole Punching/Bonding to Glass

75. Remove chips from the oven and punch appropriate holes with the hole puncher.
76. Clean glass slides with IPA and PDMS chips with Scotch tape.
77. Use plasma bonder to bond together chips and glass slides (25 s).
78. Cure at 80° C. in oven overnight.

The total curing time at 80° C. should equal at least 5 days before testing of chips, and chips should be 12 days old and autoclaved before use for cell culture.

Microfluidic Cell Culture

Microfluidic devices were placed inside a custom environmental chamber (Live Cell Instrument™, Chamlide). The temperature was maintained at 37° C. with 5% CO_2 in humidified air. Humidity saturation was maintained by the addition of two 3 cm-petri dishes filled with water inside the microscope incubator. The iso-osmotic bath and the device were filled with medium 24 hours prior to loading the cells to create equilibrium with the environment. Positive pressure was maintained by gravity in the iso-osmotic bath by connecting a 3 mL syringe filled with medium to the bath, thus preventing the formation of air bubbles that could alter imaging (see FIG. **5**). The content of the bath was replaced before cell loading but was not exchanged during the experiment. Assuming a relative humidity of 90% in the microscope incubator, we calculated the water losses from the bath to be in the order of ~1% over the course of a 5-d experiment. Water vapor loss from the osmotic bath may be modeled as a near-Fickian diffusion and has a flux given by,

$$J=-D\nabla C \quad (1)$$

where D is the diffusion constant of water vapor in PDMS (~8.5×10^{-10} m^2 s^{-1}) and C is the concentration of water vapor in the bulk PDMS. The iso-osmotic bath covers the area of the array (20 mm×11 mm) and has a height of ~5 mm. The majority of vapor loss occurs through the top surface of the chamber that is sealed with a 1 mm thick layer of PDMS and through the long and short sides of the bath that are sealed with 5 mm and 3 mm thick edges of PDMS respectively. This is well approximated as a one-dimensional diffusion for problem given by,

$$J=-D\Delta C/L \quad (2)$$

where L is the thickness of the PDMS sealing the top and 4 sides of the osmotic bath. A saturated water vapor concentration at 37° C. on the inside surface of the membrane is assumed (~39.3 mol m^{-3}). Assuming a 90% relative humidity in the incubator, the water vapor concentration at the outside surface of the chip is approximated to be 0.9×mol m^{-3}=35.4 mol m^{-3}, giving a total vapor flux of 2.1×10^{-8} g s^{-1}. This corresponds to a loss of 13 μl over a 5 day experiment. Given a total osmotic bath volume of 1.1 ml this results in approximately 1.2% change in osmotic strength during an experiment. Cells were concentrated to 2×10^6 cells/mL, transferred to a Teflon® tube and plugged in the device with a stainless steel pin.

The channels were flushed with medium and cells were pumped into the device at a rate of 1 μL/min. Cells were allowed to settle down in the chambers, then more cells were introduced until an adequate density was reached. In order to prevent air bubbles from forming inside the device, an inlet pressure of 4 psi and an outlet pressure of 1 psi were maintained at all times. When activated, pumps and valves were pressurized at 35 psi.

For cultures of ND13 and NA10hd cells, filtered DMEM with 15% FBS, 1.6 μg ml-1 puromycin, 100 ng ml-1 mouse SF, 10 ng ml-1 human IL-6 and 6 ng ml-1 mouse IL-3 (all cytokines from STEMCELL Technologies™) was exchanged by replacing four times the volume of the chip. Tests with fluorescent dye showed that this amount was sufficient to replace the volume of the chip. Referring to FIG. **19**, a microfluidic cell culture array was loaded with medium supplemented with PE-TexasRed-streptavidin and the inlet was replaced by medium only. Pictures of the last 3 columns of the array were taken during perfusion and fluorescence intensity was quantified with Image J (National Institute of Health—Collins, T. J. BioTechniques 43 (1 Suppl): 25-30 (2007)). This demonstrates that perfusing 4-fold the volume of the array (26 μl) is sufficient for complete medium exchange. Each data point in FIG. **19** represents the average of 9 wells and error bars represent the standard deviation. Despite the low flow rates, the small length of the chambers allowed for efficient exchange of nutrients, growth factors and metabolites through a combination of convection and diffusion. For small molecules (diffusion coefficient (D) was ~10^{-9} m^2 s^{-1}) or proteins (D was ~10^{-10} m^2 s^{-1}), this diffusion time was approximated by τ of ~x2/D, where x is one half the chamber height, giving exchange times of 10 s or 100 s, respectively. These exchange times are substantially shorter than the 10-15 min periods used for medium perfusion.

Medium was exchanged by replacing 3 times the volume of the chip after 24, 36, 48, 54, 60, 66 and 72 hours of

culture. For single-cell cultures of ND13 cells, we found that medium exchanges at 24, 36, 48, 54, 60, 66 and 72 h were sufficient to avoid conditions that led to decreased growth rates (owing to nutrient limitations and/or build-up of growth-inhibiting metabolites). Integrated micro-pumps and micro-valves were automatically controlled by custom scripts (LabVIEW™, National Instruments). The average doubling time (τd) for each clone was calculated by $\tau d = 72 \times \ln(2)/\ln(N72)$, where N72 is the number of cells per clone at 72 h. Primary E-SLAM cells were isolated as described previously (Kent, D. G. et al. Blood 113:6342-6350 (2009)) and cultured in Iscove modified Dulbecco medium supplemented with 10 mg ml-1 bovine serum albumin, 10 μg ml^{-1} insulin, 200 μg ml^{-1} transferrin, 40 μg ml-1 low-density lipoproteins, 100 U ml^{-1} penicillin, 100 μg ml^{-1} streptomycin, 2 mM glutamine (all from STEMCELL Technologies™), 10^{-4} M β-mercaptoethanol (Sigma™) plus 20 ng ml^{-1} IL-11 (Genetics Institute) and SF, as indicated. Before starting the experiment, the volume needed to completely exchange the medium in the array (including the dead volume from the medium inlets to the multiplexer) was tested using a fluorescent dye. Ten minutes of perfusion was sufficient to remove the dye below detectable levels. Medium was exchanged every 2 h, and we pumped for 15 min for each condition to ensure that any medium remaining from a previous condition would be washed out of the array. Images were taken every 12 min in two focal planes. Cell survival and early division times were assessed manually by looking at the videos, and the individual growth curves for each clone were generated using the bifocal image analysis algorithm described below. The content of the bath was replaced before cell loading but was not exchanged during the experiment. Assuming a relative humidity of 90% in the microscope incubator, the water losses from the bath were calculated to be in the order of ~1% over the course of a 5-day experiment.

Image Acquisition

The environmental chamber and the microfluidic devices were mounted onto an inverted microscope (Axiovert 200™, Carl Zeiss™). Bright field images were acquired with a 20× objective and a CCD camera (Orca ER, Hamamatsu) connected to a computer. The entire microfluidic cell culture array was automatically scanned with a motorized staged (ProScan II™, Prior Scientific) every 6 hours or selected wells were imaged every 5 min.

Alignment and Autofocus

Chamber alignment and autofocus scripts were implemented to acquire homogeneous images, which in turn, improved the efficiency of cell segmentation. Each of the 400 image frames contained 4 chambers. The coordinates of the 4 corners of the array were first determined manually; then coordinates for the entire grid were automatically calculated by extrapolation based on the device geometry. In order to adjust for small, local device distortions introduced during device fabrication, each image frame was automatically aligned and focused. For each image frame, both a row and column average was calculated. The dark edges of the chambers produced reproducible valleys in these profiles. The locations of these valleys were then found and used to calculate the shift needed in order to align the wells to the image. Once cells were loaded into the device, the images were automatically focused by minimizing the variance of the intensity of the pixels contained within each chamber. A constant offset was then applied to each focus position to increase the accuracy of the cell segmentation algorithm. These scripts were implemented in LabVIEW™ (National Instruments).

Image Analysis

To manage the large number of images generated per experiment, a custom image analysis software program was developed to automatically count the cells at each time point in individual chambers. Cell segmentation scripts were written in MATLAB™ (MathWorks™). Referring to FIG. **17***a*, segmentation was accomplished through three main steps: chamber segmentation (A-E), cell-containing region segmentation (F-J), and then single cell isolation (K-O). First, the individual chambers are segmented from the image background. This step of the segmentation is accomplished by applying a bandpass filter (B) and then creating a binary image through an automatically determined threshold (C). The resulting binary image is enhanced by removing objects touching the image borders and suppressing noise by removing small objects (D). Finally, the chambers are segmented from the rest of the background by filling in the holes created by the edges of the chambers. Next, the regions containing cells are separated from the rest of the chamber. This is achieved by first applying a local standard deviation filter to enhance the highly variable regions (G). The noise in the filter response is then suppressed by removing small regions, and this result is converted into a binary image through an empirically determined threshold (H). Any holes in this result are then filled in to create the final region mask (I). To segment the individual cells from the rest of the group, a bandpass filter is applied to the output of a local standard deviation filter applied to the image (K). A top hat filter is then used to enhance the edges (L), and the bounded regions are subsequently filled (M). This result is then converted to a binary image using an automatically determined threshold, and further enhanced by removing small objects (N). FIG. **17***b* shows the results of a comparison between automated and manual cell counts, which demonstrated that the automated cell count was in agreement with the manual quantification of the cells The straight line represents the 1:1 slope. Deviations at higher cell numbers are caused by the shadow around the edges some chambers, thereby resulting in a slight underestimate of cell numbers using the image algorithm. An enhanced bifocal algorithm can correct this error.

For experiments requiring a high count accuracy, for instance to generate growth curves of primary HSCs, an enhanced cell-segmentation algorithm was developed based on sets of images taken at two different focal points (~50 μm apart). One image remained in focus, and the other was taken above the focal plane for use in segmentation. After segmenting the well as described above, the portion of the image that was hidden by edge shadows was identified by comparing the intensity of the region inside the perimeter to the global mean intensity of the well. The shadow was removed by calculating a brightness gradient mask around the obstructed region, combining it with the well mask and applying it to the original image. Next, the high-contrast image was used to identify the center of cells, which appeared as high-intensity spots, by applying a brightness threshold. The centers were then dilated to achieve accurate cell size representation. The focused image was used to identify cell boundaries. The image intensity was inverted and sharpened using a negative Laplacian filter to enhance the cell edges. The sharpened image was then subtracted from the original, leaving only the cell contours and well. A bandpass size filter was then applied to remove objects that did not correspond to cell perimeters. The mask containing the cell contours was combined with the cell center mask, and the image was dilated. A watershed cut algorithm was then applied to separate adjacent cells that may have been

connected during the dilation and filling processes. Finally, the segmented image was compared to an initial image without cells, and objects common to both were removed. This enhanced bifocal algorithm gave high-accuracy cell counts with excellent correspondence to cell counts with excellent correspondence to cell counts determined by manual counting, as demonstrated in FIGS. **20***a* and **20***b*. FIG. **20***a* shows a comparison between automated and manual cell counts. The straight line corresponds to a linear least square regression. FIG. **20***b* shows absolute differences between the algorithm and manual counts.

Live Cell Immunostaining

For live cell immunostaining, the microincubator was turned off, and the main body containing the microfluidic device was placed on ice. For each step, at least ~26 μL (4-fold the volume of the entire array) was pumped into the array to ensure complete replacement of the solution. The device was filled with blocking solution for 20 min. The biotinylated antibody cocktail (anti-B220, Gr-1, and Mac-1-biotin) was then pumped into the device followed by incubation of the device for 40 min, and was then flushed with a solution of Hank's Balanced Salt Solution supplemented with 2% fetal bovine serum (2% Hanks). A PE-Texas-Red-streptavidin solution was then pumped into the device, which was then incubated for another 40 minutes, and flushed again with 2% Hanks until all background fluorescence had disappeared. The array was then filled with fresh medium and placed on the microscope for imaging. Bright field and fluorescent images (exposure time, 1 second) were taken for the entire array.

Cell Recovery

Micropipettes were pulled from glass capillaries to a diameter ranging between 80 to 140 μm. At the end of an experiment, the cover layer was delaminated from the chip, and selected colonies were recovered by piercing the membrane with a micropipette. To recover the entire content of the microfluidic device, the chip was flipped upside down and flushed with medium by pumping backwards at a rate of 1 μL/min. Cells were then recovered from the Teflon® tube and placed in a tissue culture plate for further analysis. To assess the efficiency of recovery, the plate was centrifuged for 5 min at 400 g, the cells were allowed to settle for 1 hour and then manually counted using an inverted microscope.

Macroscale Cultures

ND13 cells (Pineault, N. et al. Leukemia 19, 636-643 (2005)) were cultured in the same medium as in the microfluidic device (e.g. DMEM with 15% fetal bovine serum supplemented with growth factors (100 ng/mL murine stem cell factor, 10 ng/mL human interleukin-6, 6 ng/mL murine interleukin-3 and selected by puromycin). Cells were passaged every 2-3 days and kept in culture for at most 60 days post-infection. Control growth curves were generated with the help of an automated cell culture analyzer (Cedex™, Innovatis™). For single cell control cultures, cells were diluted to a concentration of 5 cells/mL, and separated in 200 μL cultures in a U-shaped 96-well plate. Cells were centrifuged at 400 g for 5 minutes and allowed to settle for one hour in the incubator. Wells containing single cells to start with were counted manually every 12 hours. For colony-forming cell assays, approximately 720 cells (corresponding to 11 starting cell equivalents) were recovered from the microfluidic array or conventional 96-well plates after 72 h in culture and plated into triplicate methylcellulose assays for 14 d (MethoCult 3484™, STEMCELL Technologies™), after which the number of colonies obtained was manually counted under a microscope.

In vivo hematopoietic reconstitution assays. Bone marrow cells obtained from C57B1/6Ly-Pep3b mice were highly enriched (~50% purity) for HSCs (Kent, D. G. et al. *Blood* 113, 6342-6350 (2009)), and a total of 50 cells (representing 25 HSCs) were retrovirally transduced with a NUP98-HOXA10hd retroviral vector and cultured for 11 days as previously described in Ohta, H. et al. (*Experimental Hematology* 35, 817-830 (2007)). On day 11, the cells were harvested and split equally between cultures in a 96 well dish (control) or a microfluidic array for a further 3 days of culture. Cells were harvested from both conditions, and then fractions representing $1/1{,}520^{th}$ or $1/15{,}200^{th}$ of the starting cells (estimated as a limiting dose of HSCs assuming a minimum of 60-fold or 600-fold expansion during the culture period respectively) were transplanted into lethally irradiated (810 cGy of x-rays) C57B1/6-C2J mice along with 100,000 BM helper cells. Six weeks, and 3 and 5 months later, peripheral blood samples obtained from each recipient were analyzed for evidence of donor-derived (GFP^+) lymphoid and/or myeloid cells as follows. Erythrocytes were lysed with ammonium chloride (STEMCELL™) and leukocytes were suspended in 2% Hanks (STEMCELL™) and then incubated with a combination of PE-labeled anti-Ly6G/Mac-1, perCP-Cy5.5-labelled anti-B220 and APC-labeled anti-CD4/CD8 (BD Pharmingen™). Flow cytometric analysis was then performed on a FACSAria (Becton-Dickinson™)

Transport Equations for Mathematical Modeling. The simulation of the system was performed with a three-dimensional, steady state, single phase, laminar flow model. The CFD (computational fluid dynamics) simulation has been done using FLUENT™ 6.3.26 (Fluent Inc.™). In laminar flow the Navier-Stokes equations describe the momentum transport. Therefore, the conservation of momentum in the micro-bioreactor is described by Eq. (3)

$$\frac{\partial}{\partial t}(\rho\vec{V}) + \chi\cdot(\rho\vec{V}\vec{V}) = -\nabla P + \nabla\cdot\bar{\bar{\tau}} \tag{3}$$

The conservation of mass is described by the continuity equation as follows,

$$\frac{\partial\rho}{\partial t} + \nabla\cdot\left(\rho\vec{V}\right) = 0 \tag{4}$$

where ρ (Kg m-3) is the fluid density, $\vec{V}$ (m s-1) is the velocity vector of the fluid, P (Pa) is the pressure, and $\bar{\tau}$ is the stress tensor. Water has been used as a model to estimate the physical properties of fluid at 37° C.

Boundary Conditions for Mathematical Modeling

The uniform velocity profile has been defined as the inlet boundary condition. At the outflow boundary, the diffusion fluxes for all flow variables in the direction normal to the exit plane are assumed to be zero. The fluid temperature is assumed to be constant at 37° C., and a no-slip boundary condition has been specified for the velocity at the walls.

Statistical Analysis

Error bars were calculated using s.d. of the mean. Relative risk and 95% confidence intervals for the Cox proportional hazard model were calculated using the 'coxph' function from the R package 'survival' with tied times of death being handled using the Efron approximation.

EXAMPLES

Example 1.1

Design of a Microfluidic Device for Suspension Cell Culture

Referring to FIG. **1**, a schematic drawing of a microfluidic device according to one embodiment is shown generally at **10**, with micrographs as insets. The microfluidice device **10** comprises an array of 1,600 chambers **12**, each having a volume of 4.1 nL with integrated microvalves to allow precise control and exchange of media. The chambers **12** are connected by flow channels **14**. Hydration lines **16** are located on each side of the array to minimize edge effects. Control lines consist of an isolation valve **18** and control lines (for example, a peristaltic pump) **20** to control cell loading and perfusion rates. Fluid can be introduced to the microfluidic device **10** through an array inlet **22** in order to access the flow channels **14** and chambers **12**. Fluid may leave the device through an array outlet **24**. Arrows point at single cells. The left scale bar represents 1 mm and the right scale bar represents 100 μm. Alternative embodiments could contain 1 to 50,000 chambers with volumes ranging from 1 nL to 20 μL. Alternatively, if one large chamber was connected by flow channels on top the volume may be about 5 mL. Chamber geometries exploit the properties of laminar flow to allow for immobilization of non-adherent cells without significant mechanical stress during and between medium exchanges. Various embodiments of the device also allow facile and efficient recovery of the pooled or individual contents of the chambers.

In order to exploit microfabrication methods that allow dense integration of microvalves (Unger, M. A. et al. Science 288, 113-116 (2000); Duffy, D. C. et al. Analytical Chemistry 70, 4974-4984 (1998); Thorsen, T. et al. Science 298, 580-584, doi:10.1126/science.1076996 (2002)), PDMS was chosen as a preferred material. Other biocompatible polymers such as poly(methyl methacrylate) (PMMA), poly (L-lactic-coglycolic acid) (PLGA) or poly(glycerol sebacate) (PGS) PDMS could also be used for fabricating similar devices. In addition it will be appreciated by one skilled in the art that other materials such as alternative elastomers, polymers, semiconductors, or glass could be used. FIG. **2** is a schematic diagram of the layers that are assembled during the fabrication of microfluidic device. The previously mentioned problems of microfluidic devices made from PDMS were address by incorporating an integrated iso-osmotic bath **32** into the design of microfluidic device. This was achieved by fabricating the nanovolume chambers in the chamber layer (cell culture array) **38** and control structures in the control layer **36** under a 150 μm thick PDMS membrane **34** that separates them from an "iso-osmotic bath" **32** consisting of a macroscopic chamber filled with medium (~750 μL in volume) and enclosed by a gas-permeable PDMS cover layer **30** to keep the bath sterile. The cell culture layer, control layer and membrane were bound to each other by multilayer soft lithography while the membrane, iso-osmotic reservoir **32** and cover layer **30** were assembled through PDMS stamping. The PDMS chips were then bound to a glass slide **40**. The integrated iso-osmotic bath **32** reservoir was filled with medium to prevent evaporation and maintain constant osmolarity inside the chambers. The iso-osmotic bath can be filled with medium and pressurized by gravity to avoid formation of air bubbles. The bath can, in some examples, be scaled proportionally to fit the area of the cell culture array, and the membrane can range from less than 1 μm to 5 mm thick depending on the application and the choice of material. The relatively high volume ratio of the osmotic bath to the culture volume (~100 times) and the lower surface to volume ratio of the osmotic bath as well as the near-saturation humidity provided by the microscope incubator, together allow a preferred osmotic strength to be maintained in each microculture for many days. Continuous exchange through the membrane also keeps PDMS-permeable medium components in equilibrium and dilutes any potentially toxic organic molecules into the large volume of the osmotic bath (Regehr, K. J. et al. Lab on a Chip 9, 2132-2139, doi:10.1039/b903043c (2009)). In a an embodiment a static bath may be used. However, smaller bath volumes could also be used if the bath content was exchanged frequently. This could for instance be done using channels overlaying the chambers that are refreshed with new medium. In an embodiment illustrated in FIG. **5**, the bath content can be replaced by removing a bath plug **13** and introducing fresh medium from a pump, such as syringe **19**, which may connect to the array via bath inlet **17**. In this embodiment, the array inlet **22** and array outlet **24** are pressurized by air and control lines **20** are connected to solenoid actuators and rest on a glass plate **40**.

Example 1.2

Cell Immobilization

Various embodiments allow for perfusion of cells without disturbing cell position. This capability may be exploited for experiments requiring dynamic medium exchange or immunolabeling of the cells during or at the end of an experiment. This is particularly useful for suspension cells.

Referring to FIG. **3**, one embodiment shows a microfluidic device is a non-perturbing microfluidic cell capture and retention mechanism that uses gravity to trap cells **50** in chambers with an inverted geometry with flow channels **14** running over the top and control lines **18**. In the disclosed embodiment, the chambers **12** have cubic dimensions of 160 μm×160 μm×160 μm. However, larger (up to 1 mm×1 mm×1 mm) or smaller (down to 10 μm×10 μm×10 μm) could be used depending on the cell type and the intended application of the device. The chambers according to this embodiment have an aspect ratio of 1:1. However, chambers having an aspect ratio as low as 0.5 may be utilized to minimize shear forces on the cells (for example, when non-adherent cells are used).

The chamber dimensions and flow rates may be designed to ensure that a permissible maximum force (for example, shear force) is exerted on cells during medium exchange and this may be adjusted as appropriate depending on the cell types being used. If cells are completely non-adhering the chambers may be designed such that the forces do not produce any significant motion. The degree of motion considered significant will be dictated by the application for which the device is being used. For example, in an imaging application or manual cell lineage analysis it may be required that the cells move less than one diameter between image capture events which may be anywhere from seconds to several hours to days. The microfluidic cell culture arrays may exploit laminar flow to deliver the cells to the chambers and then to ensure that the cells are not disturbed by subsequent perfusion. During medium refreshment or cell loading the volume expansion from the flow channels (≤13 μm×100 μm) to the chambers (160 μm×160 μm) creates a large reduction in velocity that drops off quickly to very low levels at the bottom of the culture chambers (e.g. FIGS. **3**

and **4**). In alternative embodiments where the depth of the chamber (i.e. length of the shortest distance between the retaining position and first region) was 80 μm (y) and the length of the first region was 160 μm (x), it was observed that the velocity of the perfusion fluid at the retaining position was such that the cells at the retaining position were being moved by the flow of perfusion fluid. The flow channel could be positioned at different heights within the chamber, as long as the bottom of the chamber is far enough from the channel so that the velocity at the bottom of the well is low enough to maintain cells immobilized by gravity. In the tested embodiment, the suspended cells were first loaded into the array using the microfabricated peristaltic pump **20** (FIG. **1**) at an overall flow rate of 1 μL/min. This corresponds to a maximum velocity of ~1 mm/sec and shear stresses of <0.3 Pa (not shown), which is well below levels that elicit physiological responses (Ma, N. N. et al. Biotechnology and Bioengineering 80, 428-437, doi:10.1002/bit.10387 (2002)). Syringe pumps, manual, gravity or pneumatic pressurization could be used as alternatives to the integrated micropump to control the flow rates. Both pulsatile and continuous flows may be used to ensure minimal cell motion during medium exchange. During loading, cells essentially follow the streamlines at the top of the chambers, and thus pass through the array without having the time to settle in the chambers. Once the array is filled, the flow is stopped, and this then allows the cells to settle into the bottom of the chambers where they are sequestered from the flow streamlines. When necessary, cells may be concentrated on the chip by repeating this loading process in a step-wise fashion. Typical loading efficiencies of 10-30% of chambers may be achieved for clonal analyses (i.e., approximately 160-480 single cells per device). A person of skill would be able to direct the desired number of cells to a chamber by adjusting cell concentrations, flow rates, flow times, etc. Cell trapping cups could be integrated in the flow channels to increase the seeding efficiency in other embodiments. Additionally, other trapping mechanisms, including dielectric forces, magnetic forces, and optical forces could be used as appropriate. Alternatively, valve structures could be designed to deterministically place cells in chambers.

In the preferred embodiment, medium exchange through the array at a flow rate of 2 μL/min, results in a maximum shear stress $<10^{-4}$ Pa at a distance of one cell diameter from the chamber bottom (not shown). Direct observation of cells in arrays being perfused at this rate to exchange media or for immunolabeling showed that the positions of the cells remained undisturbed (FIG. **9***a*), thereby validating the use of these strategies while monitoring the growth of individual clones. This capability is demonstrated in FIG. **9***b* where frequent imaging (<5 min) was used to track the progeny of three single HSCs and build cell lineage trees over 60 hours while replacing the cell culture media every 6 hours (see FIG. **9***c*). The frequency of image acquisition can be adjusted based on the number of wells being observed and the time required to capture images of all the chambers.

In an embodiment described herein, the small chamber length-scale allows for efficient exchange of nutrients, growth factors and metabolites by a combination of convection and diffusion. For small molecules ($D\sim10^{-9}$ m^2/sec) or proteins ($D\sim10^{-10}$ m^2/sec), this diffusion time is approximated by $\tau\sim x^2/D$ where x is one half the chamber height, giving exchange times of 10 sec or 100 sec, respectively. This is significantly shorter than our medium perfusion protocols that have refresh times of 10 minutes.

Recovery of Cells Post-culture

Cell recovery is often required to enable functional assays to be performed on the progeny of the input cells, or to select cells of interest for larger scale culture. A method to recover defined clonal populations is therefore a critical requirement for many applications of microfluidic cultures.

FIG. **4** shows a numerical simulation of the flow profile through a culture chamber of an embodiment. With a flow rate of 0.0625 μl/min through the flow channel, the velocity in mm s^{-1} for the flow channel **14** is 2.4 mm s^{-1} at the center of the flow channel, with a gradual decrease at the edges of the flow channel to about 1.0-1.2 mm s^{-1}. The sudden expansion when the fluid moves from the flow channel to the chamber creates a velocity drop, and the velocity in the cell retaining region is reduced to less than 50 μm/s. The velocity in mm s^{-1} for the culture chamber **12** ranges from about 1.6 to about 0.4 mm s^{-1} immediately adjacent the inlet and outlet (see bright flares) of the flow channel and the remainder of the culture chamber **12** ranges from about 0.4 to about 0.0 mm s^{-1}. The culture chamber **12** dimensions (160 μm×160 μm), flow channel **14** dimensions (100 μm×13 μm) and culture chamber **12** volume (4.1 nl) are also shown. The modeling predicts minimal flow rates at the bottom 5/6 of the chamber. In accordance with embodiments, the gravitational forces on the cells is greater than hydrodynamic forces and cells remain in the cell retaining region while the perfusion fluid exits the chamber through the flow channel outlet. Similarly, modeling of fluid velocity during cell loading (modeled for a total flow rate of 1 μL/min) suggests that a maximum velocity in the flow channels **14** does not exceed 1.2×10^{-3} m/s and that the maximum velocity in the majority of the chamber **12** is at or near 0 m/s (not shown) during cell loading. When the flow is stopped, cells settle down in the chamber **12** by gravity to the cell retaining region. Additionally, modeling of the sheer stress (Pa) on the channel walls **14** suggests during cell loading, the flow rate of 0.03 μl/min through the flow channel results in a maximum shear stress exerted on the cells is 0.3 Pa next to the channel wall (not shown). Similarly, modeling of sheer stress on cells within a chamber **12** during media exchange (i.e. perfusion) at a flow rate of 0.0625 μl/min through the flow channel suggests that the maximum shear exerted on the cells while at the bottom of the chamber (i.e. cell retaining region) during medium exchange (based on a total flow rate of 2 μl min^{-1}) does not exceed 3.1×10^{-4} Pa and would be about 0.0004 Pa in the middle of the cell retaining region.

Embodiments of the chamber design and microfluidic apparatus described also allow for facile recovery of cells from the entire array by simply inverting the device, causing the cells to settle into the higher-flow rate regions of the chambers (as shown in FIG. **4**) and then recovering the pooled population by flushing back through the input port. This recovery method is simple and efficient, allowing for the harvesting of approximately 90% of cells with losses mainly attributable to the nonspecific adherence of cells on the surface of chambers. However, when selective recovery of the contents of specific individual wells is desired, the layer of PDMS covering the osmotic bath can first be removed and a sterile micropipette then used to pierce the membrane over any selected chamber followed by aspiration of its contents (not shown). This method was found to be remarkably reliable and easy, allowing more than 90% of the cells in each well harvested to be recovered as determined by direct cell counts before and after (FIGS. **9***a* and **9***b*). It can be performed either manually of automatically if greater throughput is needed. Furthermore, aspiration through a

capillary (controlled with micron precision) or optical forces could be used to recover select cells. Alternatively, cells could be labeled individually using optical means while in the device, recovered together by flowing out of the device, and then subsequently identified using the marker. One such marker may be a fluorophore that changes spectral properties when illuminated by a light source such as a focused laser or other light source capable of selectively labeling cells.

Example 1.3

Culture of Hematopoietic Cells

Culture of single hematopoietic cells in microfluidic cell culture arrays. We tested the applicability of this microfluidic device to the study primitive hematopoietic cells. We first examined the growth of a preleukemic murine cells created by genetically engineering primitive adult mouse bone marrow cells to express a NUP98-HOXD13 (ND13) fusion gene[9,10]. Matched cultures of these "ND13" cells were set up in 24-well plates seeded at 150,000 cells/mL, 96-well plates seeded with single cells, and microfluidic cell culture arrays with or without the integrated iso-osmotic bath. In the presence of the iso-osmotic bath, the population doubling time averaged over all chambers loaded with single cells faithfully reproduced the bulk growth rate seen in the culture plates, indicating comparable conditions had been achieved. In addition, the average rates of expansion of the clones generated in the microfluidic chambers were equivalent to the average growth rates obtained in the 200 μl 96-well cultures (FIG. **10**). However, in devices that lacked the iso-osmotic bath, cell division and survival was severely compromised (e.g. FIG. **10**), in spite of humidity control in the microscope incubator and the initiation of medium exchanges 24 hours after starting the experiment, indicating that permeation effects occur within hours, which can in turn affect cell growth. In cases where other materials are used that have reduced transport properties the osmotic bath may not be required. Alternatively, the use of perfusion with sufficient frequency may be used to reduce the need for the osmotic bath.

A single cell in a 4.1 nL isolated chamber is at an effective concentration ~2.5×10^5 cells/mL. At confluence, a chamber contains ~150 cells; i.e., a concentration of ~4×10^7 cells/mL. This concentration greatly exceeds the limits of conventional batch cultures. Thus, it is not surprising that cultures exhibited a strongly inverse correlation between the number of cells inoculated into each isolated chamber and the duration of cell growth, in the absence of the iso-osmotic bath batch mode (FIG. **11**). This underscores the importance of medium exchange to sustain the continued optimal growth of these cells in nanolitre-volume chambers, and the need to progressively increase the frequency of medium exchange as the number of cells in each culture increases. For the single ND13 cell cultures, we found that medium exchanges at 24, 36, 48, 54, 60, 66 and 72 hours were sufficient to avoid nutrient limitations and the build-up of growth-inhibiting metabolites, although this feeding pattern can be adjusted based on cell types, seeding density and required nutrient concentrations.

Example 1.4

Assessment of Growth Heterogeneity by Defined Cell Populations

Time-lapse imaging and automated image analysis was used to generate individual growth curves for 243 single ND13 cells over a period of 72 hours (a sample is shown in FIG. **12***a*). After that time, the fastest growing clones became multilayered and too large for further tracking by image analysis. Although the average doubling time for all cells was 16.8 hours, we observed substantial heterogeneity in the growth characteristics of individual clones. 52% of the input cells either did not divide or produced progeny that died before the end of the experiment. This widespread death was offset by the rapid proliferation of other cells that divided as frequently as every 12 hours, but with large variability between clones (FIGS. **12***b* and **12***c*). Such variable clone size distribution was also observed for ND13 cells generating clones in the 96 well plate cultures (not shown). Furthermore, similar distributions of doubling times were found for both ND13 cells grown in microfluidic arrays and multiwell macro plate controls (not shown). A similar experiment was conducted using normal primary HSCs. Microfluidic culture of freshly isolated CD45+EPCR+ CD48−CD150+ (E-SLAM) adult mouse bone marrow cells, which are approximately 50% pure HSCs (Schroeder, T. Cell Stem Cell 1, 479-481 (2007)), over 5 days showed that the kinetics of three successive divisions were comparable to those obtained in macroscale cultures (Dykstra, B. et al. Proc. Natl. Acad. Sci. USA 103, 8185-8190 (2006); Kent, D. G. et al. Blood 112 , 560-567 (2008)) (see FIG. **21**).

To further investigate this heterogeneity, we stained ND13 cells for the lineage (lin) markers (Gr-1, Mac-1, and B-220) and compared the clonal growth kinetics of single differentiated (lin^+) and primitive (lin^-) cells. We opted for lineage staining to characterize cells in this particular experiment but antibody staining, enzymatic assays, dyes, RT-qPCR, sequencing, functional assays, or bead capture could also be used to characterize the cells. After staining, cells were introduced into the device and imaged every 5 minutes for 72 hours. We used the perfusion capabilities to perform a second lineage staining on clones at the end of the experiment without disturbing colony locations. This experiment showed that most of the lin^+ cells did not produce colonies (FIG. **13***a*), which replicated the failure of lin^+ cells to form colonies in 96-well cultures. In contrast, the single lin^- cells produced clones efficiently but of different sizes and phenotypes (FIG. **13***b*). Some of the lin^- cells gave rise to exclusively lin^+ or lin^- clones. In other cases, the clones were of mixed phenotypes (FIG. **13***c*). This suggests that ND13 cells maintain a lin^- clonogenic progenitor population that can produce both more of themselves (i.e., lin^- cells) as well as more mature non-clonogenic lin^+ cells. Further support for this model was obtained by isolating lin^- cells by FACS, expanding them in macroscale cultures, and then demonstrating after 12 days that a new lin^+ population had again been produced (FIG. **18**).

In a different experiment, unseparated ND13 cells were cultured in the microfluidic device for 72 hours and then approximately 720 cells from 5 chambers (corresponding to 11 starting cell equivalents) recovered (FIG. **13***d*) and plated into triplicate colony-forming cell assays in methylcellulose-containing medium. Parallel methylcellulose assays were set up with the progeny of 11 starting cell equivalents generated in standard control cultures. The number of colonies obtained from each source was again similar, further demonstrating the equivalence of the microfluidic device in supporting ND13 progenitor expansion (FIG. **13***d*).

Example 1.5

Expansion of HSCs

To test the suitability of these microfluidic cell culture arrays to support HSC self-renewal divisions, we examined

the growth of mouse bone marrow cells transduced with a NUP98-HOXA10homeodomain (NA10hd) fusion gene, which potently stimulates their ability to expand in vitro without any signs of leukemic transformation (Ohta, H. et al. Experimental Hematology 35, 817-830, doi:10.1016/j.exphem.2007.02.012 (2007); and Pineault, N. et al. Molecular and Cellular Biology 24, 1907-1917 (2004)). To obtain these cells, we first isolated a highly purified population of primary HSCs (with a $CD45^+CD48^-EPCR^+CD150^+$ phenotype), and then transduced these cells with a NA10hd-encoding retroviral vector. The transduced cells were then expanded for 11 days in a macroscale culture. At the end of this period, replicate aliquots were then transferred either to the microfluidic array or a control macroscale vessel and cultured for an additional 60 hours. The cells from each of these latter cultures were then recovered and decreasing fractions of the same starting equivalent number injected into groups of 6 mice each. The total number of cells obtained from the chip and the control macrocultures were similar (FIG. **14***a*). All mice showed similar reconstitution levels by the transplanted cells for >16 weeks post-transplant, indicative of an overall stem cell expansion of more than 600-fold compared to the stem cell content of the purified cells initially transduced (FIG. **14***b*). The mice repopulated with cells from the microfluidic array also showed reconstitution of both their myeloid and lymphoid compartments (FIG. **15***a*). Notably, the cultured NA10 HSC population contained a greater proportion of fast growing cells compared to the ND13 cells (FIG. **12***b*), consistent with the lack of highly mature cells in the NA10 population.

Example 1.6

HSC Response to Temporally Varied SF Stimulation

Previous work has shown that in vitro exposure of HSCs to low concentrations of steel factor (SF) (1 ng ml^{-1}) leads to rapid loss of HSC function, delayed proliferation and increased death compared to culture in higher concentrations (300 ng ml^{-1}) (Kent 2008 supra). However, the reversibility of the effect of low SF concentrations on HSC survival and proliferation is not known. To address this question microfluidic system as described herein were used to test how long quiescent adult HSCs could be exposed to a low SF concentration before rescue by exposure to a high concentration was no longer possible. An enlarged microfluidic device consisting of 6,144 individual chambers and additional inlets and flow control valves to enable parallel studies with many temporally varied conditions was used (FIG. **16**). Six different conditions in which primary mouse HSCs (E-SLAM isolates of adult mouse bone marrow) were exposed to 20 ng ml^{-1} of interleukin-11 (IL-11) plus either 1 ng ml^{-1} SF for the first 8, 16, 24 or 48 h followed by 300 ng ml^{-1} SF for the remainder of the experiment, or constant SF concentrations of 1 ng ml^{-1} or 300 ng ml^{-1} for the entire experiment (not shown). The experiment was repeated twice yielding 5 days of imaging data for a total of 769 single E-SLAM cells cultured in the device. By day 5, the fastest growing clones reached confluence, and we could no longer quantitatively monitor their size. Growth rates of all clones were compared to the results for the constant high SF concentration. As a control, the same cells were grown in conventional macrocultures in 20 ng ml^{-1} IL-11 plus either 1 ng ml^{-1} or 300 ng ml^{-1} SF and these yielded the same growth kinetics as in the microfluidic device. Compared to the high [SF] condition, a Cox proportional hazard analysis of the cell survival over time, defined as the fraction of starting cells that remained viable or gave rise to clones, showed no significant difference (P>0.1) in survival when the cells were rescued from 1 ng ml^{-1} SF exposure within the first 16 h of culture (Table 1).

TABLE 1

Cox proportional hazard analysis of mouse HSC survival

Condition	n	Relative risk (95% CI)	P value
High [SF] (300 ng ml−1)	294	1.00	—
8 h in low [SF]	107	0.82 (0.64-1.06)	0.13
16 h in low [SF]	76	1.03 (0.78-1.36)	0.84
24 h in low [SF]	24	1.27 (0.81-1.99)	0.29
48 h in low [SF]	79	1.78 (1.37-2.31)	<0.0001
Low [SF] (1 ng ml−1)	189	1.53 (1.25-1.86)	<0.0001

CI, confidence interval. —, not applicable. Relative risks and P values were calculated based on the high [SF] condition.

Prolonged initial exposure to 1 ng ml^{-1} SF led to a rapid decrease in viability between 16 and 24 h, and after that time, the cells could not be rescued by exposure to 300 ng ml^{-1} SF (FIG. **6**). Most dividing cells completed a first mitosis between 24 and 60 h of culture for all conditions, and the SF concentration did not affect the cell division kinetics (FIG. **7** and FIG. **8**). Analysis of the second division showed comparable kinetics, with more than 80% of the clones remaining viable after a first division regardless of the SF concentration to which they had been initially exposed. Thus, although a high SF concentration influenced the viability of HSCs as they exited quiescence, it did not directly impact the subsequent division kinetics of cells that complete a first division.

Microfluidic technology brings the potential of chemical control of the culture medium in combination with single cell imaging to create new opportunities for the high throughput analysis of clonogenic cell responses to varying extracellular cues. The embodiments described herein introduce several design features which enable experiments with heterogeneous populations of suspension cells, even those that have stringent medium requirements. Some particular embodiments may include the incorporation of an enclosed and sterile iso-osmotic reservoir to control unwanted permeation and dehydration effects separated by a membrane from high aspect ratio wells to contain and immobilize non-adherent test cells during perfusion, and the use of a reverse perfusion strategy or selective aspiration to recover all cells or selected clones.

Using aspects and embodiments described herein, it is demonstrated herein that successful culture of cytokine-dependent hematopoietic cells is possible with expansion and enhanced HSC function. Maintaining equilibrium with the macroscopic volume of the osmotic bath allows for high-throughput microfluidic single cell cultures in volumes that are 4 orders of magnitude smaller than conventional macroscale cultures. A single cell isolated in a 4 nL chamber is at an effective density of 2.5×10^5/mL, thus making possible the investigation of autocrine signaling by isolated cells, with the potential of increasing plating efficiency for cell types that might otherwise require conditioned medium or a high cell density. Co-culture of different cell types at limiting dilution could further be used to investigate the effect of cell-cell influences through secreted factors. It is

also worth noting that with sufficient medium exchange, we were able to maintain cell proliferation to densities that resulted in the creation of multiple layers of cells. This ability to maintain high-density cultures offers new opportunities for studying the effects of cell concentration on cell behavior.

Various embodiments also provide flexibility to monitor the clonal growth (or other responses) of single non-adherent cells over time in the presence of dynamic changes in medium conditions by combined time-lapse imaging with programmed medium exchanges that do not disturb the spatial position of each cell or colony. It has been shown, for instance, that exposure of HSC in vitro to sub-optimal steel factor concentrations can induce their differentiation within 16 hours even prior to their entry into the cell cycle (Kent, D. G. et al. Blood 112, 560-567, doi:10.1182/blood-2007-10-117820 (2008)). Thus, it is relevant to anticipate that other schedules of growth factor delivery can further modulate HSC fate decisions. The fully programmable system for both perfusion and image acquisition allows automated and dynamic temporal operation over the entire duration of the culture experiment, thereby providing a mean to analyze the evolution of clonal cultures in time rather than measuring only end-point outcomes. In addition, the ability to replace the culture medium is a key feature to avoid nutrient limitations that occur in longer-term experiments in which even a small amount of proliferation causes a significant increase in the local cell concentration.

Imaging the cellular contents of 1,600 chambers requires less than 5 minutes allowing the changes to be monitored at high temporal resolution. When coupled with emerging image processing tools for identifying new morphological phenotypes (Cohen, A. R. et al. Nat Meth 7, 213-218 (2010)) and for tracking different cell divisions identified by specific markers or fluorescent reporters (Eilken, H. M. et al., Nature 457, 896-900 (2009); and Satyanarayana, S. et al. Journal of Microelectromechanical Systems 14, 392-399 (2005)), the combined advantages of high throughput and medium control will now allow previously impossible large-scale studies of fate-choices by rare cell types.

The growth kinetics analysis performed on the ND13 population yielded findings that can only be revealed from clonal analyses. The scale of the perfusion microfluidic cell culture array described here was purposefully optimized for the study of small numbers of hematopoietic cells, but can be readily modified to give designs with other features; e.g. more or larger chambers. Situations where only a small fraction of the cells are responsible for the long-term maintenance of the overall population are not exclusive to the hematopoietic system. The technology is highly suitable for adaptation to other cell types/organisms and other applications such as drug-response screens, culture optimization, clone selection, recombinant protein production and cell characterization. Various aspects and embodiments described herein are also ideally suited to controlled experiments investigating the interaction of two or more cell types. The extended use of microfluidic systems coupled with live-cell microscopy thus offers great promise for many applications of scientific investigation in biology and medicine.

What is claimed is:

1. A method of culturing a cell, the method comprising:

retaining the cell at a retaining position within an individual chamber of a microfabricated device;

perfusing the cell with a perfusion fluid by flowing the perfusion fluid into the individual chamber through an inlet and out of the chamber through an outlet,

wherein the outlet is positioned such that gravitational forces acting on the cell to keep it at or near the retaining position exceed hydrodynamic forces acting on the cell to move it toward the outlet;

culturing the cell within the chamber and monitoring a response in the chamber; and

selectively recovering the cell or a clonal population thereof from the individual chamber based on the response in the monitoring step.

2. The method of claim **1**, further comprising regulating osmolarity of the perfusion fluid within the chamber.

3. The method of claim **2**, wherein regulating osmolarity of the perfusion fluid within the chamber comprises placing the chamber in gaseous communication with a bathing fluid, wherein the bathing fluid has a volume greater than the chamber volume.

4. The method of claim **3**, wherein the bathing fluid and the perfusion fluid are iso-osmotic.

5. The method of claim **1**, wherein a speed of the perfusion fluid is decreased to less than 50 μm/s as the perfusion fluid approaches the retaining position.

6. The method of claim **5**, wherein the speed of the perfusion fluid is decreased to about 0 μm/s as the perfusion fluid approaches the retaining position.

7. The method of claim **1**, wherein the chamber has a top and a bottom, and the retaining position is at the bottom.

8. The method of claim **7**, wherein the inlet position is proximal to the top.

9. The method of claim **7** or **8**, wherein the outlet position is proximal to the top.

10. The method of claim **1**, wherein the cell is a suspension cell.

11. The method of claim **1**, wherein the perfusion fluid comprises any one or more of a cell culture medium, an immunostaining agent, an enzymatic reagent, a dye, a buffer, an oil, and a bead-containing solution.

12. The method of claim **1**, wherein x is less than or equal to y and x is the length of the shortest distance between the inlet and the outlet and y is the length of the shortest distance between the retaining position and a region of the chamber that is interposed directly between the inlet and outlet positions.

13. The method of claim **12**, wherein the ratio of x:y of the chamber is greater than 0.5.

14. The method of claim **1**, further comprising a step of flowing the cell into the chamber prior to retaining the cell at the retaining position.

15. The method of claim **1**, further comprising isolating a clone of the cell.

16. The method of claim **1**, further comprising tracking the progeny of the cell.

17. The method of claim **1**, wherein the flow of the perfusing fluid is intermittent.

18. The method of claim **1**, wherein the flowing of the perfusing fluid is continuous.

19. The method of claim **1**, further comprising characterizing the cells by lineage staining, antibody staining, enzymatic assaying, RT-PCR analysis, sequencing, functional assaying, or bead capturing to characterize the cells.

20. The method of claim **19**, further comprising selecting cell clones based on said characterizing.

21. The method of claim **2**, wherein the bathing fluid is located in a reservoir, wherein the reservoir is separated from the chamber by a gas-permeable polydimethylsiloxane (PDMS) layer.

22. The method of claim 2, wherein regulating osmolarity within the chamber comprises placing the chamber in gaseous communication with an osmolarity regulator.

23. The method of claim 22, wherein the osmolarity regulator comprises a vapor and gas-permeable PDMS layer.

24. The method of claim 1, wherein the chamber volume is less than about 10 nL.

25. The method of claim 24, wherein the chamber volume is about 4.1 nL.

26. The method of claim 1, further comprising imaging the chamber to obtain an image of the chamber comprising the cell.

27. The method of claim 26, further comprising analyzing the image of the chamber to determine a cellular response.

28. The method of claim 1, wherein the cellular response is growth rate kinetics.

29. The method of claim 1, wherein the cellular response is cell death.

30. The method of claim 1, wherein the cellular response is determined using a fluorescent reporter.

31. The method of claim 1, wherein recovering the cell comprises piercing the chamber and aspirating the chamber's contents or a portion thereof to obtain the recovered cell.

32. The method of claim 1, wherein the inlet is operable as the outlet.

33. The method of claim 1, wherein the outlet is operable as the inlet.

34. The method of claim 1, further comprising exchanging the perfusion fluid.

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FORM 19. Certificate of Compliance with Type-Volume Limitations **Form 19**
July 2020

UNITED STATES COURT OF APPEALS
FOR THE FEDERAL CIRCUIT

CERTIFICATE OF COMPLIANCE WITH TYPE-VOLUME LIMITATIONS

Case Number: 23-2227

Short Case Caption: Bruker Cellular Analysis, Inc. v. University of British Columbia

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